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Comparison of Microshear Bond Strength and Morphological Changes Between Active and Passive Application of 4th Generation Etch-and-Rinse Etchant on Enamel

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Comparison of Microshear Bond Strength and Morphological Changes Between Active
and Passive Application of 4th Generation Etch-and-Rinse Etchant on Enamel

by

Sophia Tuyet-Nhi Trieu

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
Biology

Thesis Committee:
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Portland State University
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ABSTRACT

Over 200 million dental restorations are performed each year in America. A dental restoration require a strong bonding of restoration to tooth structure and relies on the dental adhesive to create this mechanical and chemical bonding. Dental adhesion or bonding is the process of forming an adhesive joint between the composite and tooth substrate: dentin or enamel. Clinical problems such as microleakage at the restoration tooth interface, influx of fluids, or bacteria growth at the cavity wall can be prevented with adhesives that obtain a more intimate bonding. Longevity of the restoration can be enhanced by the adhesive that creates the tight bonding to reduce problems such as postoperative sensitivity, marginal staining, and recurrent caries. The goal of this research project is to investigate the influence of active scrubbing application as compared to passive non-scrubbing application of the etchant component in 4th generation etch-and-rinse adhesive systems. Shear bond stresses have been measured and compared between application techniques. Verification of resin infiltration depth with each etchant application has been examined with scanning electron microscopy by mounting the etched and bonded enamel surface of the tooth in epoxy and slicing the tooth longitudinally producing a transverse, depth-wise view. Results from this study have clarified the role of resin tag formation as well as tooth morphology during an active acid etchant application for dental restoration.

Dedication

*To my supportive and loving parents, Le Kim Xuan & Trieu Sanh, who have been better
parents than I could have ever wished for.*

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GLOSSARY

1. Dental restoration: when correction or repair of malformed, damaged, or missing tooth structure is fixed clinically, a dental restorative material is used to restore the function, integrity, and morphology of the missing tooth structure.
2. Resin composite: a dental restoration material made of synthetic resin, usually acrylic base, to which a high percentage of inert filler has been added, can be made to match the patient's tooth color and is used as the chief filling material in dental restorations.
3. Etching: preparation of tooth surface and dental materials with etching materials, usually phosphoric acid, is done to roughen the surface and increase adhesion.
4. Dental adhesive: act as the adherent between the tooth structure and the dental restoration material.
5. Adhesive systems: currently there are 4 dental adhesive systems in the market: 4th – 7th generations. The 4th and 5th generations belong to the etch-and-rinse systems which have a separate etching step and the 6th and 7th generations belong to the self-etch systems which do not have a separate etching step.
6. Shear strength: describes the strength of a material against the structural failure where the component fails in shear. Shear strength is measured by $T = F \text{ (force)} / A \text{ (area)}$.
7. Tensile strength: the maximum strength a material can withstand when subjected to tension.
8. Failure site: observation of the type of failure at the site where bond strength was tested.
9. Failure site category: i) adhesive interface between tooth and adhesive (A), ii) mixed with tooth and adhesive and/or composite resin (M), iii) cohesive within tooth (Co-T), or iv) cohesive within the composite (Co-C).

The National Institute of Dental Research reported that approximately 94% of adults in the United States show evidence of past or present dental caries and estimate that approximately 61.6 million adults could benefit from professional dental restoration (Brown, Winn et al. 1996). Placement and replacement of composite restorations constitute approximately 60% of all operative dentistry done (Mjor 1998). Dental adhesion or bonding is the process of forming an adhesive joint between the composite and tooth substrate: dentin or enamel. Clinical problems such as microleakage at the restoration tooth interface, influx of fluids, or bacteria growth at the cavity wall can be prevented with adhesives that obtain a more intimate bonding between the restorative material and the tooth (Perdiago 2007). Longevity of the restoration can be enhanced by adhesives that create the secure adhesion to reduce problems such as postoperative sensitivity, marginal staining, and recurrent caries (Duke 1993).

Several studies have demonstrated that active application as opposed to passive application of certain adhesive systems or adhesive components enhances adhesion (Jacobsen and Soderholm 1998; Salz, Zimmermann et al. 2005; Dal-Biano, Pellizzaro et al. 2006; Reis, Pellazaro et al. 2007; Higashi, Michel et al. 2009). The enhancement of adhesion or the quality of bond strength may be determined through a variety of methods included tensile or shear-bond strength testing, resin infiltration, microleakage evaluation, thermocycling, and observations of changes in tooth surfaces such as the etch pattern produced by the dental adhesive. Although enamel bonding has improved

substantially with the use of appropriate adhesives there are still times when bond failure occurs (Swift, Perdigao et al. 1995). This has facilitated a strong motive to find ways of maximizing the bond to minimize failures. Many dental adhesives have been formulated; currently on the market are the 4-7th generation adhesive systems. The gold standard for dental adhesives is the 4th generation etch-and-rinse system which gives the strongest bond strength. On the other hand, there is no information regarding the effect of actively vs. passively applying etchant onto enamel using 4th generation etch-and-rinse adhesive systems.

The goal of this research project was to investigate the influence of active (scrubbing with a micro brush) application as compared to passive (non-scrubbing) application of the etchant component in 4th generation etch-and-rinse adhesive systems. Microshear bond test was implemented for this project since this is a well-tested and commonly utilized method for screening the effectiveness of factors on adhesion. Restoration bond strength was tested using microshear bond testing. Evaluation of etch pattern and resin infiltration at the micro level have been captured with SEM images for further analysis of the etch pattern between treatment groups of active and passive application of etchant. Verification of differences in etch pattern examining resin infiltration depth and etch morphology patterns have been observed with SEM images.

DENTAL RESTORATION

Dental restorations are important for our oral health by allowing reconstruction of tooth with dental materials. It is estimated that over 60 million people in the United States could benefit from dental restorations (Brown, Winn et al. 1996). Therefore, there is a strong appeal in the advancement of dental restorations which comprises about 60% of treatment accomplished by operative dentists (Reinhard 2001).

The advantages of dental restorations were originally intended to restore tooth structure, fractures, or to fill tooth erosion and abrasive defects (Kidd 1976). This purpose still holds true today but has expanded its scope toward other avenues in operative dentistry from preventative treatments to esthetic restorations. In traditional operative dentistry a restoration would often require the removal of sound tooth structure in order to gain retention and stabilization of the dental restoration material. Modern dentistry adopted the use of adhesives, which reduce the size of tooth preparation which was a momentous advancement in the field of operative dentistry (Buonocore 1955; Fusayama, Nakamura et al. 1979).

Adhesives are agents that help the restoration adhere to the tooth structure. As such, adhesives are the bonding intermediate, acting as an adherent between two interfaces. Dental restorations can be complex with many interfaces joining together, such as the enamel-adhesive-composite-adhesive-porcelain interface in a porcelain

restoration (Perdiago 2007). Adhesives play a critical role in significantly increasing the bond strength of the restoration to tooth structure. Tooth preparation can also be minimized with adhesives since the etch pattern increases surface area and allows better restoration retention without the need remove sound tooth structure to provide macromechanical retention features. The more secure bonding with adhesives decreases microleakage where recurring caries occurring from an ingress of fluids will damage the tooth and restoration (Duke 1993).

Today, esthetic dentistry has become very appealing for the general population and many people demand esthetic, reliable restorations and are willing to pay for cosmetic reconstruction work. Matching an individual's tooth color is made possible with the use of resin filled adhesives that can match the individual dentition. For that reason, adhesives are useful by allowing tooth colored restorative materials to esthetically restore or recontour teeth relatively easily and economically (Strassler 1991). Adhesives entail relatively simple steps and provide patients with dependable restorations with a natural appearance.

The use of phosphoric acid to improve the bonding mechanism of resin to enamel was established by Bunocore in 1955 when he applied the idea of industrial use of phosphoric acid to improve adhesion of paints to resin coatings (Buonocore 1955). He revolutionized the practice of restorative dentistry and made adhesives a common step in restorative dentistry. Bunocore discovered that 85% phosphoric acid made acrylic resin adhere more securely to an etched enamel surface. He demonstrated that acid etching of enamel with phosphoric acid increased the bond strength 100-fold.

Today, adhesives are commonly used in restorative dentistry and advancements to improve current products are being researched. Whereas the traditional restoration concepts contended with large tooth preparations to retain restorative materials, contemporary restorations are able to minimize tooth preparations with the use of adhesives (Black 1917). Adhesives allow a more intimate bond of the restoration to tooth structure that will inevitably reduce microleakage and increase restoration longevity. Reducing microleakage will decrease clinical problems such as recurrent caries, marginal staining, and postoperative sensitivity (Duke 1993).

Traditional metal restorations requiring removal of sound tooth for mechanical retention can weaken the tooth's infrastructure by acting as a wedge between the lingual and buccal walls and increasing the risk of cuspal fracture. On the contrary, a weakened tooth structure can be reinforced by adhesive restoration since it can better transmit and distribute functional stress across a bonded interface (Morin, DeLong et al. 1984; Eakle 1986). Restoring teeth with little or no tooth preparation is made possible by dental adhesives and not only strengthens a weakened tooth but can also salvage a carious tooth. Thus, adhesive give dentists the technology to provide a good restoration with durability, longevity, and esthetics.

Dental adhesives work by fundamentally exchanging the inorganic tooth material with synthetic resin where the tooth-composite interface attains a tight mechanical and chemical interlocking (Van Meerbeek 2001). Bonding systems have an acid component that will partially demineralize the cut dentin or enamel surface for resin monomers to attain optimal tooth infiltration (Pashley, Ciucchi et al. 1993). The adhesive component

is a solution of resin monomers that provide a micromechanical interlocking retention between the two substrates upon polymerization. The first step is to remove superficial calcium phosphate, also known as hydroxyapatite, to expose microporosities in dentin and enamel. The second step, hybridization phase, involves a resin infiltration and polymerization into the microporosities created by the etchant (Perdiago 2007). Therefore, adhesives allow a mechanical interlocking essential to a sealed restoration with minimal invasion into sound tooth structure.

This micromechanical interlocking is the special feature that increases bond strength by creating a larger surface area for more adherence of resin monomers. Calcium phosphate is removed revealing an etchant pattern of an irregular surface with microporosities and deep grooves where resin monomers can more effectively infiltrate into etched tooth surface (Swift 1995). The last step in adhesive bonding is the hybridization phase which allows deeper resin infiltration and more surface area contact that inevitably increases micromechanical bonding. The prepared tooth surface will now readily bond securely with resin monomers from the restoration material. Therefore, adhesives are critical in the improved retention of restorative material to the tooth surface but most importantly it helps to seal the margins which remains to a major obstacle in clinical longevity (Gaengler, Hoyer et al. 2004).

ENAMEL AND DENTIN STRUCTURE

The composition of enamel and dentin are very different, but modern adhesive systems are able to achieve acceptable bonding effectiveness when they are simultaneously applied to both enamel and dentin (Bertolotti 1991). Nonetheless, enamel and dentin have different bonding properties due to their physiochemical and structural differences. Enamel is comprised of 86 % of inorganic content by volume, primarily hydroxyapatite, and 2 vol% organic material with water comprising the last 12 vol% (Gwinnett 1990). On the other hand, dentin is made up of 50 vol% inorganic material

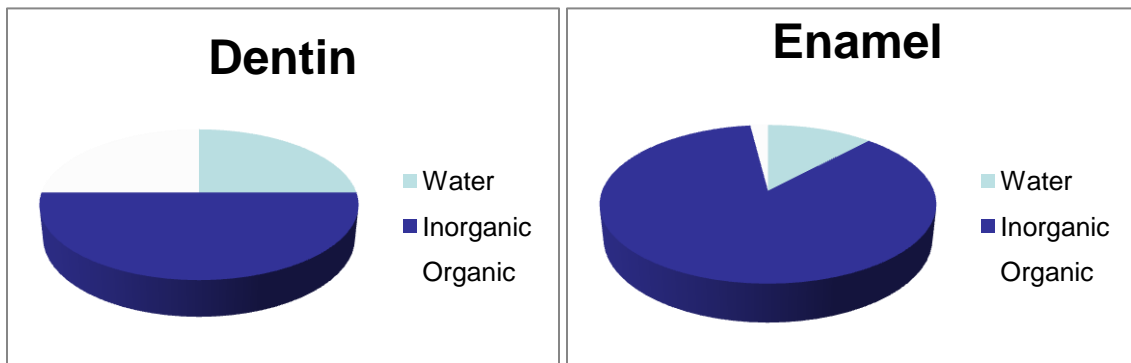


Figure 1: Enamel and dentin diagram illustrating the content of water, inorganic, and organic material. and 25 vol% organic collagen while containing approximately 25 vol% water (Figure 1) (Heymann 1993). Dentin is intrinsically saturated with water and has a higher outward pressure from the pulp as compared to enamel and render the two tooth tissues very different (Kerdvongbundit, Thiradilok et al. 2004). Due to its highly hydrated nature it is more difficult to adhere to dentin as compared to enamel which has less water, higher surface energy, and more inorganic material which is more appropriate for efficient bonding with bonding systems that contain hydrophobic resins (Nordenvall, Brannstrom et al. 1980).

Dentists prepare a tooth using a bur or other instrument and the residual debris forms a smear layer on the cut tooth surface (Bowen 1984). The smear layer is composed of hydroxyapatite, altered collagen, and a gellike collagen which all stand as a physical barrier for adhesive resin and bonding agent to directly contact tooth structure (Eick, Cobb et al. 1991). Debris covers the surface of enamel and dentin and needs to be dissolved or made permeable so the resin monomers in the adhesive can infiltrate to the tooth surface. For that reason, either acid or another form of etchant is applied so that resin bonding monomers can bypass the residual components of debris and penetrate deep into the tooth structure. Thus, the acid conditioning objectives are to remove the smear layer and to make enamel and dentin surfaces more receptive for bonding.

For adhesives to adequately bond to the tooth surface they need

to have intimate contact as well as sufficient wetting (Figure 2). The surface tension of the adhesive will need to be less than that of surface energy of the tooth structure for adequate wetting to occur (Erickson 1992). Enamel is comprised primarily of hydroxyapatite and has a high surface energy whereas dentin has a higher organic content causing it to have low surface energy. Thus, an intermediate resin primer is required to unite the tooth substrate to resin bonding agent.

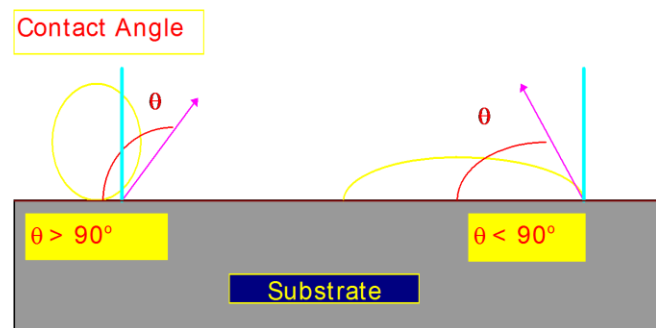


Figure 2: Example of surface wetting. Wetting of a surface by a liquid is characterized by the contact angle of a droplet that is placed on the surface. Sufficient wetting happens when the adhesive has less surface tension than the surface energy of the tooth surface.

The last phase in adhesive bonding utilizes a low viscosity bonding resin to wet the high-energy surface produced by the etchant pattern or primer components. Then the tooth's capillary tension draws the bonding resin deep into the microporosities. The tooth surface is ready for bonding to the restorative material, with the adhesive resin monomers inside the microporosities, copolymerizing with the unreacted carbon-carbon double bonds in the matrix phase of the resin composite (Torstenson and Oden 1989).

Enamel is primarily comprised of a highly mineralized inorganic substrate and acid etching will substantially enlarge the surface area for bonding (Silverstone, Saxton et al. 1975; Swift 1995). It has a smooth surface and except for some aprismatic enamel it is almost homogenous in composition and nature. Most of the inorganic fraction is submicron crystallites forming three dimensional structures called rods or prisms (Gwinnett 1990). With acid etching or conditioning, the smooth enamel surface takes on an irregular pattern with increased surface area and doubles its high surface-free energy (Jendresen, Glantz et al. 1981).

About 10µm of enamel is removed by etchant and the surface area doubles by creating an irregular microporous layer from 5 to 50 µm deep (Sano, Shono et al. 1994; Van Meerbeek, Yoshida et al. 1998). When the hydroxyapatite crystals dissolve it creates an enamel etching pattern of prism cones, peripheries, and resin tags with a multitude of individual crypts for greater surface area bonding. Resin tags are categorized into two types: macrotags forming around the prism peripheries and microtags at the core of enamel prisms (Bayne, Flemming et al. 1992). Microtags form

in multitudes of crypts where bonding resin monomers can adhere to them and are probably the reason for increased bond strength.

The dentin smear layer is comprised of porous and permeable dentin submicron channels, but the buildup of debris decreases dentin permeability by covering the microporosities of intertubular dentin and plugging the collagen tubules (Pashley 1992; Eick, Robinson et al. 1993). Besides using an etchant to simultaneously remove the smear layer and demineralize the tooth surface, dentin etching also exposes the collagen fibrils for increased resin infiltration (Bowen 1984). Although the composition of enamel and dentin are very different, tooth preparation usually requires cutting into both structures and thus modern adhesives have been tailored to fit the requirements of both substrates.

DENTAL ADHESIVE SYSTEMS

Adhesives work by exchanging inorganic tooth material for resin and the degree of exchange differs among adhesive type (Tao and Pashley 1988; Van Meerbeek 2001). Contemporary adhesives are carried out in one, two, or three application steps and the different approaches are classified by: etch-and-rinse, self-etch, and glass ionomer adhesives. Etch-and-rinse and self-etch adhesives involves the application of 1) conditioner or acid etchant, 2) primer, and lastly, 3) adhesive resin.

The most conventional adhesive systems on the market today are the 4th and 5th generation etch-and-rinse systems. These adhesive systems both operate with a separate etchant step. The 4th generation system carries out each adhesive step separately whereas the 5th generation system combines the primer and adhesive steps into a single component application. The more recent self-etch systems omit the separate etchant step by combining the etchant with a primer followed by adhesive application (6th generation), or will include all adhesive steps into one application as in the 7th generation system.

The etch-and-rinse strategy uses a separate acid or conditioning step to remove the smear layer and to demineralize the more superficial hydroxyapatite crystals. A mixture of resin monomers in the primer and adhesive is applied to infiltrate the etched dental surface. The three-step etch-and-rinse adhesive systems are considered the golden standard and have demonstrated superior performance over the two-step etch-and-rinse and self-etch adhesive systems in clinical and in vitro studies (Inoue, Vargas et al. 2001; Inoue, Vargas et al. 2003).

Self-etching adhesives do not require a separate etching step, since they condition and prime tooth structure simultaneously. The self-etch components partially demineralize the hydroxyapatite layer and work to penetrate beyond the smear layer while simultaneously infiltrating the tooth structure. This resin-infiltrated layer is the hybrid zone composed of minerals and the smear layer (Van Meerbeek, De Munck et al. 2003). Self-etching adhesives are less technique sensitive and reduce application time but etching with phosphoric acid as a separate step surpasses self-etching adhesives in clinical longevity (Perdiago, Gomes et al. 2005).

FACTORS INFLUENCING ADHESIVES

The demand for less invasive and esthetically reliable dental restorations prompts further advancement in dental adhesives. Composite restorations require the etchant to remove or penetrate the smear layer and expose more tooth surface to induce micromechanical retention. The depth of tooth structure being removed during the etching procedure depends on the type of acid, acid concentration, and the duration of acid etching as well as the chemical structure of the tooth substrate (Bates, Retief et al. 1982; Retief, Busscher et al. 1986; Bastos, Retief et al. 1988; Blosser 1990).

Several studies examined the effectiveness of phosphoric acid etching on enamel and dentin. Optimal etching with phosphoric acid has been established at a concentration between 35-40%. A study found that 35% phosphoric acid on enamel yields significantly higher bond strengths as compared to 10% maleic acid, 10% phosphoric acid, and oxalic acid/aluminum nitrate (Swift and Cloe 1993). The bond strength obtained with 35% phosphoric acid was 24.5 MPa whereas the others had considerably lower bond strengths, measuring between 6.3-13.2 MPa.

A more retentive enamel etching pattern was exhibited among self-etching adhesives when 35% phosphoric acid was applied beforehand (Rotta, Bresciani et al. 2007). Adding a separate phosphoric acid etching step also showed significantly higher microtensile bond strength (μ TBS) with Turian SPE/One-Step Plus and Clearfil SE

Bond, two self-etching adhesives that contain a milder acid concentration than other self-etch adhesives in the study conducted by Rotta, et al. The same study indicated that self-etch adhesives with stronger acids have a higher bond strength, but adding a separate etch step did not significantly improve the bond strength. Van Meerbeek further examined the effects of self-etch adhesives on enamel with additional phosphoric acid pre-treatment and stated that less marginal defects at the enamel side were noticed (Van Meerbeek, Kanumilli et al. 2005).

Further research indicates that adding a separate phosphoric acid etch prior to the application of self-etch adhesives should be limited to enamel since dentin micro-TBS was significantly decreased (Van Landyt, Kanumilli et al. 2005). They examined SEM and transmission electron microscopy (TEM) images and found that separate phosphoric acid etching on enamel results in a better micro-retentive surface. While it is important to have sufficient demineralization of dentinal tooth surface to allow adhesive penetration for hybrid layer formation there is a depth limit where too much etching will prevent the adhesive resin from reaching the bottom of the demineralized network (Wang and Spencer 2004). The reduced dentin micro-TBS with additional acid etching with self-etch adhesives is due to a poor resin infiltration of the hybrid zone (Bolanos-Carmona, Gonzalez-Lopez et al. 2008). While additional phosphoric acid application or stronger self-etch approach appears more favorable for enamel bonding, mild self-etch adhesive that leaves hydroxyapatite within a submicron hybrid layer available for additional chemical interaction provides better bonding to dentin (Moura, Pelizzaro et al. 2006).

A highly significant correlation was found between the calculated and measured depths of etch on enamel using different acid concentration and etch duration (Legler, Retief et al. 1990). This study etched the surface of ground enamel with different duration using 5, 15, and 37% phosphoric acid. The higher the concentration of acid etchant, the deeper and more pronounced the etch topography. These tests found a statistically significant linear relationship between the mean depth of the demineralized enamel layer and concentration of phosphoric acid (Holtan, Nystrom et al. 1995; Bolanos-Carmona, Gonzalez-Lopez et al. 2006). Whereas the etch duration was variable between adhesive systems and brands but all indicated an optimum etch time. Thus, they found that the etch pattern depth on enamel depended on the concentration and duration of the phosphoric acid used.

Uno and Finger conducted a study that evaluated the difference between phosphoric acid to other non-phosphoric acid etchants on enamel. They wanted to examine if those etchants would produce a highly retentive pattern and if a frosty appearance similar to clinical procedures was seen (Uno and Finger 1995). Only phosphoric acid revealed a frosty appearance as compared with alternative acids such as 10% maleic acid, 10% citric acid, 2.5% oxalic acid, and 2.5% nitric acid (Triolo, Swift et al. 1993). Some studies point to significant reduction of bond strength when there was not a frosty appearance as known with phosphoric acid (Swift and Cloe 1993), whereas other research stated that a frosty appearance does not negatively affect the adhesive bond strength (J, L et al. 1997; S and WJ 1999). At present, phosphoric acid is still the etchant of choice to attaining a strong bond to enamel (BT and J 2000).

A few studies validated manufacturer's instruction time of 15 seconds as the ideal etching time on enamel and dentin (Pioch, Stotz et al. 1998). Shorter etch times for enamel and dentin may not provide enough depth for maximum resin infiltration and result in poor bond strength. However, longer etch times for dentin produced unnecessarily deep demineralization that required deeper resin impregnation producing thicker hybrid layers that are not associated with higher bond strength. Research on etch-and-rinse adhesive systems have indicated significant differences in bond strength depending on the etch durations (Miyazaki, Platt et al. 1996). It can be concluded that adhesives can be technique sensitive where different methods can attain optimal bonding.

ADHESIVE APPLICATION METHODS: ACTIVE VS. PASSIVE ETCHING

Adhesive systems aim for close micromechanical bonding, maximum bond strength, reduced nano or microleakage, and prolonged longevity of the restoration to tooth structure. Active application of adhesives has been shown to increase bond strengths under certain conditions. Many studies have revealed that actively scrubbing the component that contains the etchant will increase bond strength of the restoration. In self-etch adhesive systems the primer contains the etchant component. Manually scrubbing the primer component of self-etch systems can provide a consistent etch and enhance the interaction of acid monomers by dispersing etching by-products on the prepared enamel surface (Miyazaki, Platt et al. 1996). This active scrubbing can disperse trapped air bubbles and mix by-products in the etchant for better removal as well as keep fresh acidic solution in contact with tooth structure for a more aggressive demineralization.

It has been made known that etch pattern and bond strengths are significantly lower with one-step adhesives as compared to total-etch adhesives (De Munck, Van Meerbeek et al. 2003). However, a study evaluating the micro-tensile bond strength (μ TBS) and nanoleakage of a 7th generation one-step self-etch adhesive system has recommended active application to improve bonding performance on dentin (do Amaral, Stanislawczuk et al. 2009). Three one-step adhesives were tested (Clearfil S³ Bond, Xeno III, and Adper Prompt L-Pop) and all showed significantly higher bond strength within a 24 hours testing time when actively scrubbing the adhesive. From the three adhesives tested with active application, two demonstrated higher bond strength and less

nanoleakage after a 6 months period in vitro. It has been suggested that agitation of the adhesive will increase water evaporation for better chemical interactions of hydrophilic and hydrophobic monomers (Tay and Pashley 2001). Passive application does not promote water evaporation which causes a poor hybrid zone with a reduced amount of resin monomers incorporated into the smear layer. This incomplete resin infiltration into demineralized dentin is a drawback with strong self-etching adhesives since longevity is jeopardized (Spencer, Wang et al. 2000).

The twofold study conducted by do Amaral showed that after 6 months the bond strengths of Adper Prompt-L-Pop when applied with or without agitation were comparable, but the treatment without agitation had significantly more nanoleakage. The continuation of dentin demineralization as seen by nanoleakage can be explained by the possibility of incomplete polymerization of monomers, which continued the process of hydrolysis after curing and additional release of phosphoric acid (Oliveira, Marshall et al. 2004). Regarding one-step self-etch adhesives, agitation by actively scrubbing onto dentin is recommended for better bonding performance and longevity.

A study comparing active agitating vs. passive application of a one-step 7th generation adhesive on enamel showed a deeper etching pattern for the active application group (Ando, Watanabe et al. 2008). SEM images indicated agitation produced a more evident etch pattern with less surface debris. It was suggested that active adhesive treatment would enhance adhesive resin penetration and achieve greater micromechanical interaction with the underlying enamel. Active agitation of one-step adhesives seems to

be encouraged for both enamel and dentin to increase bond performance and longevity as well as less degradation.

Two conjunctive studies revealed that dentin bond strengths are dependent on the pressure applied during active application of an acetone and ethanol/water based adhesive systems. Dry dentin surfaces treated with vigorous agitation had significantly increased bond strengths reaching 37.11 ± 7.3 MPa with an ethanol/water based adhesive, Single Bond (3M ESPE) and an acetone based adhesive, One-Step (Bisco). However, wet dentin surfaces only need slight agitation to attain high bond strengths (41.82 ± 8.4 MPa) since vigorous agitation gave comparable bond strengths (38.89 ± 8.2 MPa) (Dal-Biano, Pellizzaro et al. 2006; Reis, Pellazaro et al. 2007).

Acetone has been shown to be effective with wet dentin surfaces due to the “water-displacing” ability of acetone (Kanca 1992). Water-displacing raises vapor-pressure and explains some of the kinetics during primer application. Though it seems as if acetone-based solvents perform better, others have reasoned other expectations in the clinical setting. Clinically, dentin may desiccate before the primer is placed or acetone may evaporate and not provide results seen in vitro. It is critical to keep dentin moist since collapsed collagen will interfere with resin infiltration and water-based primers that keep dentin moist may be more effective in clinical settings. Accordingly, adhesive systems generate different effects with primer agitation and successful bond strength is achieved when considering acid concentration, application duration, and drying times.

Active application of the primer component on enamel with self-etching adhesives have also demonstrated higher bond strengths (Miyazaki, Hinoura et al. 2002). They concluded that agitation and drying time of primer can influence enamel bond strengths by dispersing the adhesive evenly along the tooth surface. A similar study examined ultramorphological changes after agitation of self-etching primers on enamel and found that better etch patterns were noticed with agitation of adhesive. However, active application only significantly improve bonding efficacy for some of the two-step adhesives in the study (Cehreli and Eminkahyagil 2006). Therefore, increase in bond strength on enamel with primer agitation for two-step self-etching adhesives appears to be dependent on the material used.

Miyazaki et. al went on to conduct a similar study on dentin and although bond strengths were higher when primer was agitated the results were not significant (Miyazaki, Platt et al. 1996). The effect of agitating with primer was conducted concurrently with air-drying time and there was an optimal range of drying times. It is believed that the slight increase in bond strength with agitation is probably due to diffusion of the amphiphilic monomer into the collagen mesh. Other studies were in line with these findings with agitation of water-based primer on wet or dry dentin (Jacobsen and Soderholm 1998) (Miears, Charlton et al. 1995; Finger and Uno 1996).

Velasquez et al. used a mild two-step self-etching system, Clearfil SE Bond, to find that shear bond strength to dentin improved significantly with agitation for 20 seconds, with some improvement for 10 seconds, and no difference for 30 seconds agitations (Velasquez and Sergent 2006). Finding the best application time is important

since acid monomer may not adequately etch and penetrate tooth structure, thereby producing a poor bond.

Chan and others demonstrated significantly higher bond strengths when agitating the primer component from 6th and 7th generation adhesive systems (Chan, Tay et al. 2003). SEM images indicated that actively scrubbing a thick layer of primer effectively dissolved the smear layer and created a thicker hybrid zone. Thus, technique-sensitive factors such as agitation, duration, and amount of adhesive can benefit the bond strength when using self-etching systems on dentin.

Many studies have demonstrated that achieving optimal bonding is technique sensitive and is dependent upon many factors. The presented cases have illustrated significant improvement in bond strength with active application. However, there has not been a study examining the effect of agitation of etchant on either enamel or dentin. Considering literature information, this study aims to examine the bond strength and resin infiltration when actively applying etchant on enamel.

PURPOSE AND SIGNIFICANCE OF THESIS RESEARCH

Clinical problems such as microleakage at the restoration tooth interface, influx of fluids, or bacteria growth at the cavity wall can be prevented with adhesives that obtain a more intimate bonding to the tooth structure. Longevity of the restoration can be enhanced by adhesives that create intimate bonding and thereby minimize problems such as postoperative sensitivity, marginal staining, and recurrent caries.

The goal of this research project is to investigate the influence of active application as compared to passive application of the etchant component in a 4th generation system of etch-and-rinse adhesives on enamel adhesion. Studies have shown that enamel etching which increases surface area of the tooth allows a more intimate bonding of the composite to the tooth (Swift 1995). Many studies have indicated that active application versus passive application of various adhesive components to both dentin and enamel increases bond strength (Velasquez 2006).

A hypothesized increase in adhesive bond strength by active application will be investigated by determining various factors involved in composite to tooth bonding. The statistical values and effect of active vs. passive application of etchant will be compared. The results of this study will help to clarify the clinical value as well as the tooth morphology with active acid etchant application during dental restoration preparation. Ultimately, restoration longevity will be increased if active application of etchant is a key component in increasing composite bonding to the enamel tooth structure. Specifically, I will address the following points: (1) Examine whether active application of etch on enamel will significantly increase microshear bond strength, (2) determine the mode of

failure when testing the bond strength, and (3) observe the etch pattern and analyze the differences caused by the different treatments of active scrubbing and passive application of etchant. The null hypothesis for this project states that actively applying acid etchant on enamel will not be significantly different as compared to passive treatment of etchant.

This study will address the goals of the project by the following applications. (1) Examine whether active application of etch on enamel will significantly increase microshear bond strength. Microshear bond stress will be measured and compared between enamel that was prepared with active application and with passive application of an etchant. I will apply the acid etchant with active or passive rubbing on prepared enamel surface. A test jig will be used to measure the micro shear bond stress (μ SBT) of the composite resin. (2) Determine the mode of failure when testing the bond strength. Failure mode will be examined with a scanning electron microscopy, SEM at 200x magnification and the mode of failure will be identified as the following: i) adhesive interface between tooth and adhesive (A), ii) mixed with tooth and adhesive or composite resin (M), iii) cohesive within tooth (Co-T), or vi) cohesive within the composite (Co-C). Any variation or significance of failure mode between the etch groups will be assessed. Lastly, (3) Observe etch pattern and analyze the differences caused by the different treatments of active scrubbing and passive application of etchant. I will examine the surface area after shear bond testing using a SEM at 200X and calculate the amount of resin tags remaining on the tested tooth surface with a software imaging program. Surface roughness length will be measured and compared between the two treatments. Observation in changes in surface morphology between the two treatments will be

considered in combination with surface roughness analysis. Statistical analysis will be calculated to determine the significance of active vs. passive application of etchant.

SAMPLE ACQUISITION

Human molars within six months of extraction were available through dental clinics and maintained in 0.5% chloramine T. 30 sound, non-carious and restoration free, human molars were used. 20 teeth for Part A: microshear bond strength testing, five teeth for Part B: evaluating surface morphological changes, and five teeth for Part C: resin infiltration evaluation.

The 30 teeth were randomly divided into two groups, active or passive application of etchant. One group was treated with scrubbing of acid etchant and the other group represented the control where acid application was done according to manufacturer's instructions. The buccal and lingual enamel surfaces were prepared for "cut" enamel adhesion testing.

PART A: TOOTH PREPARATION FOR MICROSHEAR BOND TEST

For microshear bond testing on enamel: the buccal and/or lingual enamel was reduced to be free of dentin or exposures. The tooth was sectioned mesial/distally to produce two pieces for testing. The tooth or tooth section was mounted with dental stone in a fixture to produce blocks with the tooth surface rising above the stone and parallel to the base.



Figure 3: Active and passive application of acid etchant onto ground enamel.

The specimens were stored in 100% humidity until testing (i.e.

within one week). “Cut” enamel surfaces were reground on 600 grit SiC paper before bonding. Surfaces were treated with Optibond FL (Kerr, Orange, CA) etch-and-rinse adhesive by either of the two methods: a) active scrubbing application or b) passive stationary application of 37.5% phosphoric acid gel for 15 seconds (Figure 3). Thus, etchant is the 37.5% phosphoric acid gel that was injected onto prepared enamel surface and was either scrubbed (medium pressure) with a flexible disposable micro-brush applicator (Kerr, Orange, CA) held at a 45° angle or applied passively for 15 seconds. Rinse time was standardize at 10 seconds per sample with a stream of water directed above the etched surface. The samples were then lightly dried for 5 seconds with an air syringe held at a distance of 10 inches. The etched enamel surface was followed by adhesive resin according to manufacturer’s instructions and irradiated for 20 seconds with a Demi LED curing unit (Kerr, Middleton, WI). The Demi LED had an output > ~700 mW/cm² and emitted from a large head allowing curing of the entire surface at one

time. Tygon ® polyvinylchloride (PVC) tubing with 1.00 mm diameter was cut into 2 mm lengths. The tubing was filled with composite, Tetric EvoFlow (Ivoclar Vivadent, Amherst, NY), and placed over prepared enamel surface and irradiated for 30 seconds according to manufacturer's instructions. Within 1 minute of curing the composite, the specimen was then stored in 100% humidity at 37°C for 24 hours and tubing was removed and specimen was subjected for microshear bond testing.

MICROSHEAR BOND TESTING

Each prepared tooth was placed into an Instron universal mechanical testing machine (Instron Corp., Boston, MA) and a max force and load data was programmed using TestWorks™ for Window (MTS Systems Corp by SINTECH, version 3.08). A shear load gripping arrangement was made where a wire loop (~0.03 mm in diameter) wraps around the specimen immediately adjacent to the composite/tooth substrate

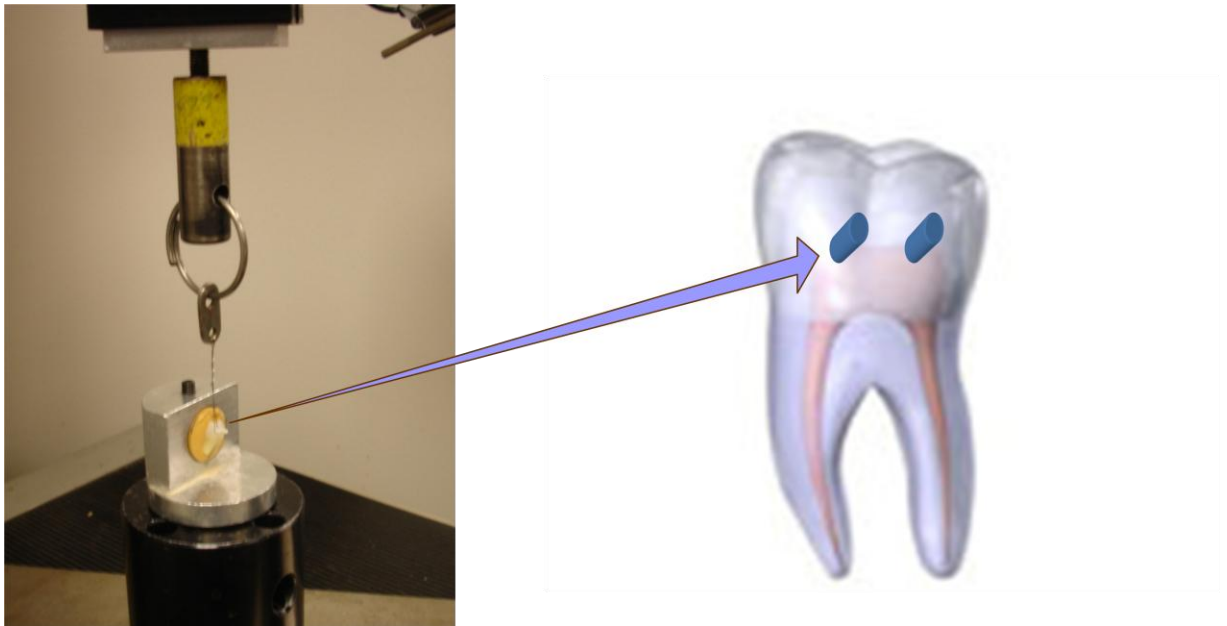


Figure 4: Test jig and shear apparatus for microshear bond testing.

Interface (Figure 4). Thus, the pull of the Instron machine was parallel to the composite specimen base. Cross-sectional area was determined by measuring the specimen area (1.00mm^2) and the failure mode was set at shear. Shear stress is computed by TestWorks™ for Window where the shear stress formula was ($\tau = \text{Force}/\text{Area}$) used to calculate the stress in kilogram and MPa. Each specimen was tested to failure in shear

mode and loaded to failure at a crosshead speed of 1 mm/minute at a 100 lb. full scale. Microshear stress was computed by dividing observed maximum load by the adhesion area ($n = 4$ to 5 tests per tooth). Shear bond stress is most appropriate for my test objective since it allows us to compare the results with other tests done in vitro and vivo. Thus, measuring shear stress can be clinically sufficient.

BOND FAILURE MODE

The failure site was evaluated with a scanning electron microscopy, SEM (Oxford Instruments, Austin, TX). The failures site was viewed at 200X magnification and the failure mode identified as: i) adhesive interface between tooth and adhesive (A), ii) mixed with tooth and adhesive or composite resin (M), iii) cohesive within tooth (Co-T), or vi) cohesive within the composite (Co-C).

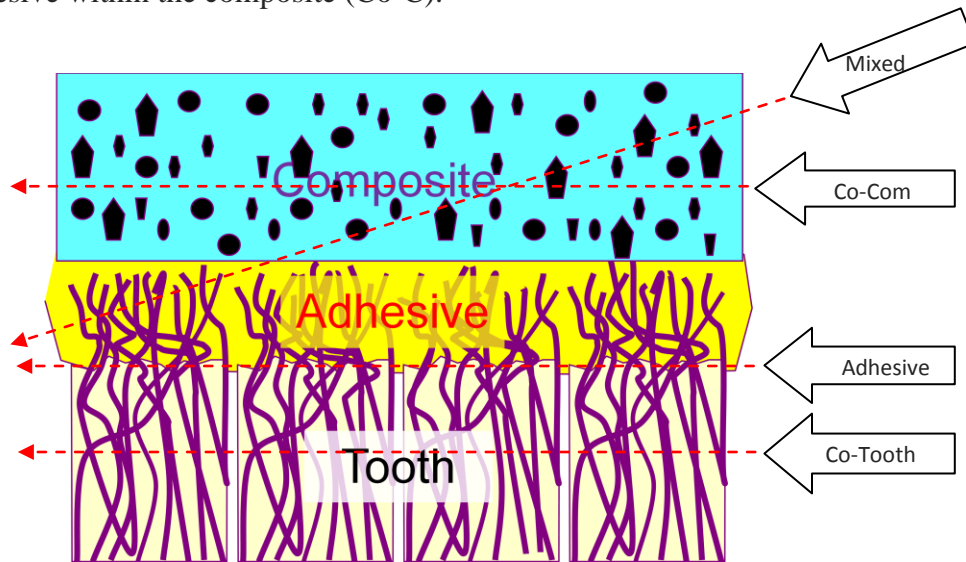


Figure 5: Illustration of failure sites for microshear bond strength.

BOND FAILURE SITE EXAMINED WITH SCANNING ELECTRON MICROSCOPY

The surface where each specimen was tested for micro-shear bond failure was further examined using a SEM. Maximized dimensions using a square image was situated within the circular facet of each failure site. To avoid ambiguity, the images were programmed to have the same spot, brightness, and contrast settings then were photographed at 200X. This SEM image size of $1 \times 1 \text{ mm}^2$ captures the 1 mm^2 radius of the specimen site. Secondary and backscatter images were used to illustrate the ratio of composite or adhesive resin to tooth structure.

The ratio of adhesive or bond resin to tooth was determined using an software imaging program (ImageJ, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA). Grey scale imaging was done to depict the difference between tooth substrate, adhesive, or bonding resin. Grey value is defined as the brightness of pixels in an image, expressed in integers ranging from 0 (black) to 255 (white) for an 8-bit digital signal. The software program computed the ratio of adhesive or bond resin to tooth structure. Failure site was then evaluated according to the same failure mode as with a stereomicroscope where: i) adhesive interface between tooth and adhesive (A), ii) mixed with tooth and adhesive or composite resin (M), iii) cohesive within tooth (Co-T), or vi) cohesive within the composite (Co-C) (Figure 6).

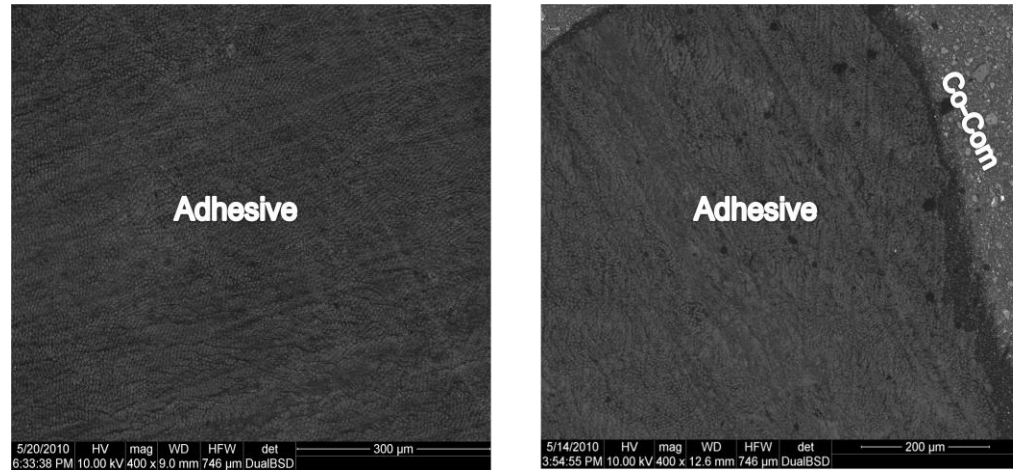
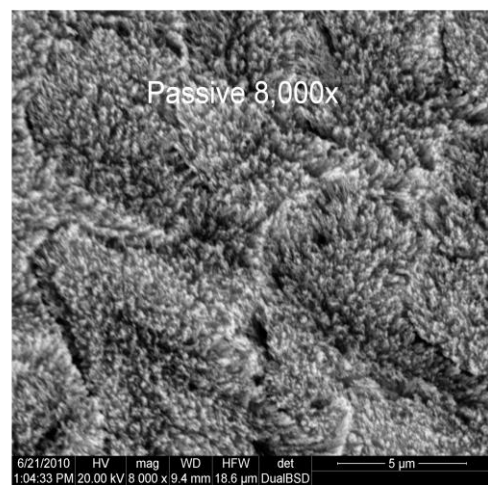
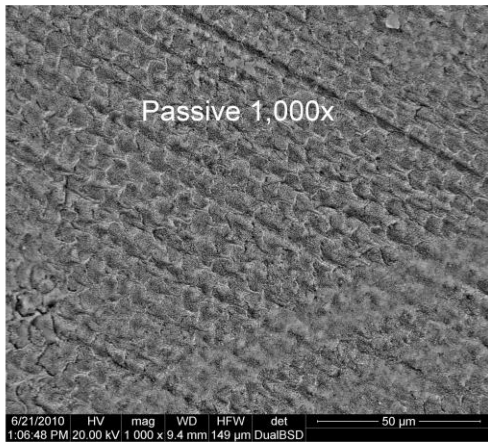
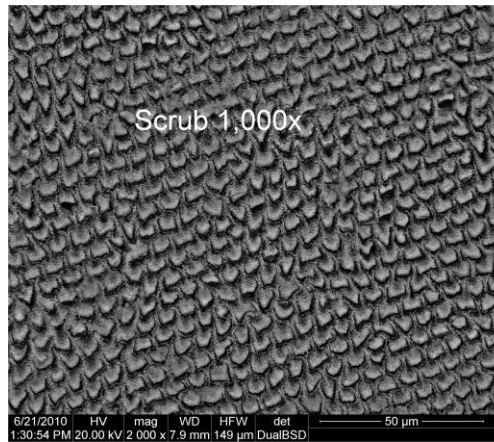
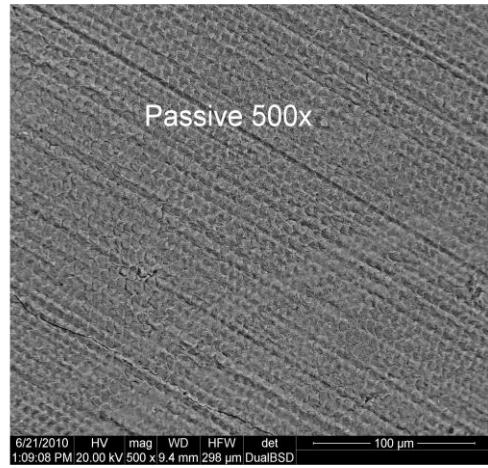
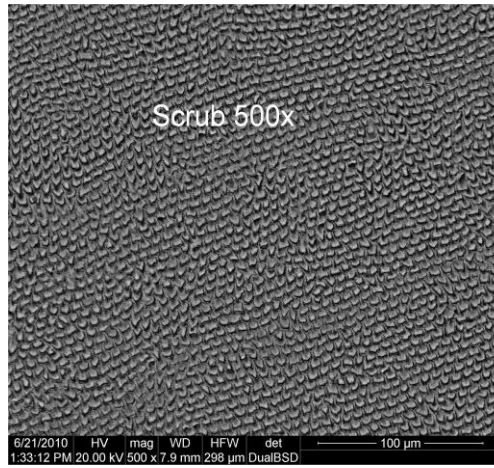


Figure 6: Example of failure site with SEM. Left image shows mixed (M) failure which is a combination of adhesive(A) and cohesive failure, in this case, cohesive within composite failure (Co-C).

PART B: TOOTH PREPARATION FOR MORPHOLOGICAL EVALUATION

For surface morphological changes on enamel: the buccal and/or lingual enamel was reduced to be free of dentin or exposures. “Cut” enamel surfaces were reground on 600 grit SiC paper before treated with etchant. The teeth were bisected mesial distally through the prepared enamel surface with a low-speed diamond saw under coolant water spray to obtain buccal and lingual halves. Each tooth halves were treated with Optibond FL (Kerr, Orange, CA) etch-and-rinse adhesive by either: a) active scrubbing application or b) passive stationary application of 37.5% phosphoric acid gel for 15 seconds. Thus, the buccal and lingual surfaces on each tooth was treated with either active or passive application of etchant where either one of its buccal or lingual surfaces was either scrubbed or not-scrubbed with etch. Rinse time was standardize at 10 seconds per sample with a stream of water directed above the etched surface and then air dried for 30 seconds. Thereafter, specimens were mounted on aluminum stubs, sputter coated gold-palladium twice for 25 seconds (Denton Vacuum Desk II sputter coater, Moorestown, NJ). Observations under scanning electron microscopy were viewed at 20kV of accelerating voltage and images were taken under 500x, 1,000x, 8,000x, and 20,000x magnification (Figure 7).



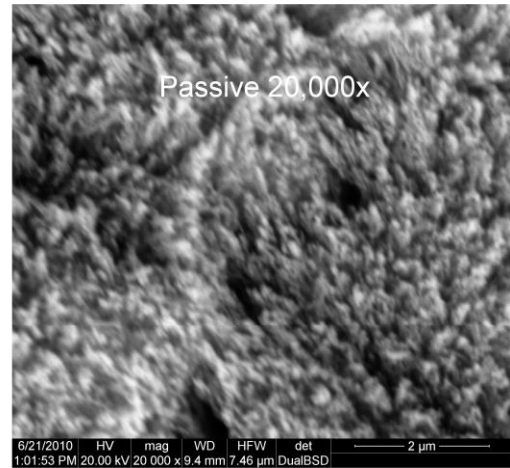
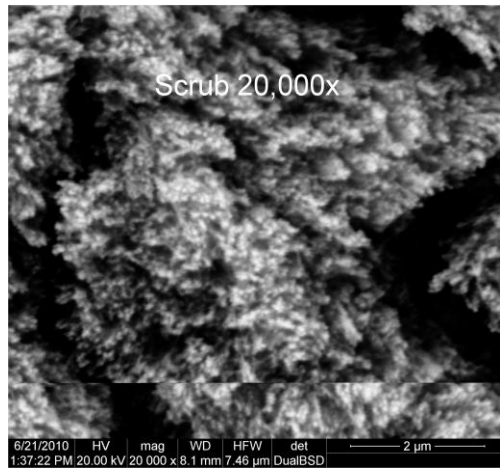
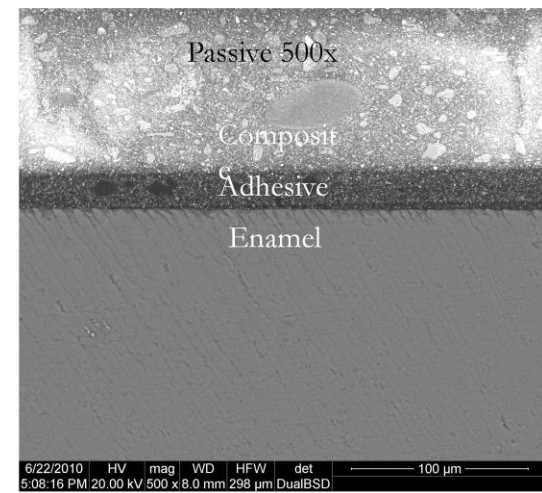
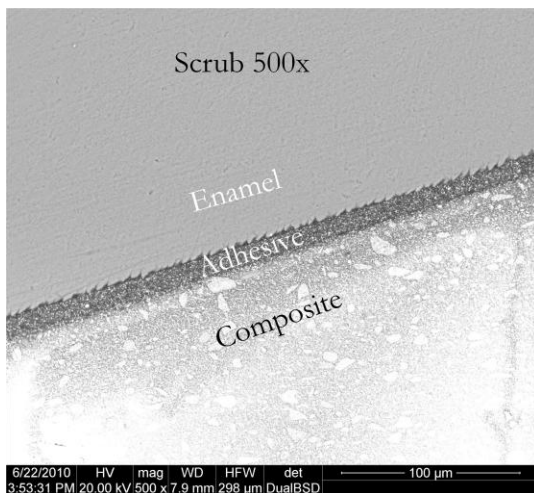
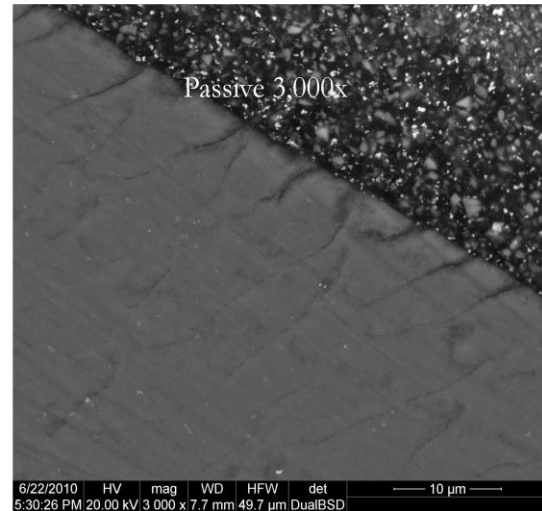
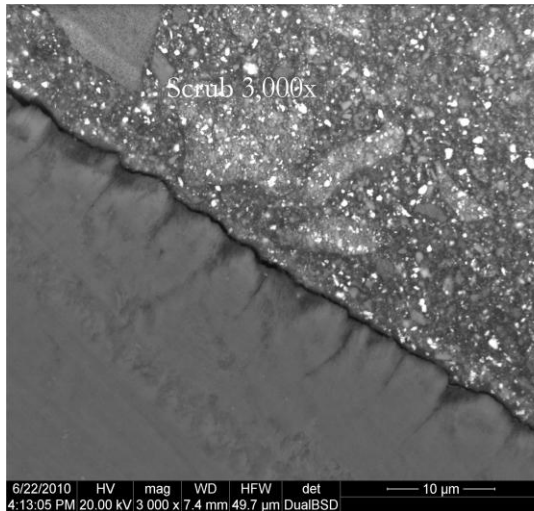


Figure 7: SEM images of enamel surface treated with scrubbing and passive application of etchant.

PART C: TOOTH PREPARATION FOR RESIN INFILTRATION

For resin infiltration evaluation on enamel: the buccal and/or lingual enamel was reduced to be free of dentin or exposures.). “Cut” enamel surfaces were reground on 600 grit SiC paper before bonding of restoration material. Each tooth was treated with Optibond FL (Kerr, Orange, CA) etch-and-rinse adhesive by both of the two methods: a) active scrubbing application or b) passive stationary application of 37.5% phosphoric acid gel for 15 seconds. Thus, the buccal and lingual surfaces on each tooth was treated with either active or passive application of etchant where either one of its buccal or lingual surfaces was either scrubbed or not-scrubbed with etch. Rinsing time was standardize at 10 seconds per sample with a stream of water directed above the etched surface. The samples were then lightly dried for 5 seconds with an air syringe held at a distance of 10 inches. The etched enamel surface was followed by adhesive resin according to manufacturer’s instructions and irradiated for 20 seconds with an Demi LED curing unit (Kerr, Middleton, WI). The Demi LED had an output $> \sim 700 \text{ mW/cm}^2$ and emitted from a large head allowing curing of the entire surface at one time. Lastly, composite, Tetric EvoFlow (Ivoclar Vivadent, Amherst, NY) was placed in one application over prepared enamel surface and irradiated for 30 seconds according to manufacturer’s instructions. The teeth were bisected buccal lingually through the prepared enamel surface with a low-speed diamond saw under coolant water spray to obtain mesial and distal halves. The cut surface was polished with 4,000 grit SiC paper that was mounted on a rotating disk with running water for 4 minutes and then air dried.

Observations under scanning electron microscopy were viewed at 20kV of accelerating voltage and images were taken under 500x, 3,000x, and 5,000x magnification.



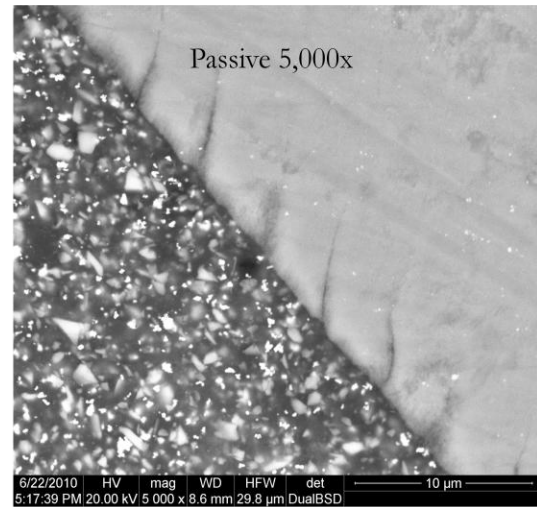
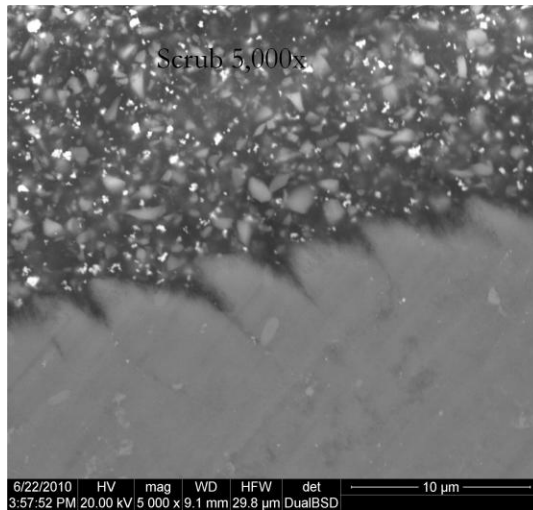


Figure 8: SEM images of cross-sectional views of a dental restoration with scrubbing and passive application of etchant.

EVALUATION OF SURFACE ROUGHNESS

Etched surfaces between the two treatments were further examined with SEM.

Cross-sectional views of the restoration were examined at the following magnifications: 500x, 3,000x, and 5,000x. To avoid ambiguity, the images were set at the midpoint of the restoration with similar brightness and contrast settings.

The surface roughness caused by the etchant was examined and measured using a software imaging program (ImageJ). Since the surface roughness causes differences in peak and valley heights within the enamel surface, length measurement of the surface roughness was done by tracing the etch pattern and comparing the length. A standard linear length of 20 μ m was used to set the dimensions of measurement within the 1,000x and 5,000x SEM images. Within the set length of 20 μ m, the tracing tool was utilized to trace and measure the surface roughness of the etched enamel surface (Figure 9). Surface roughness differences among the treatment groups and the correlation between the 3,000x and 5,000x images was recorded.

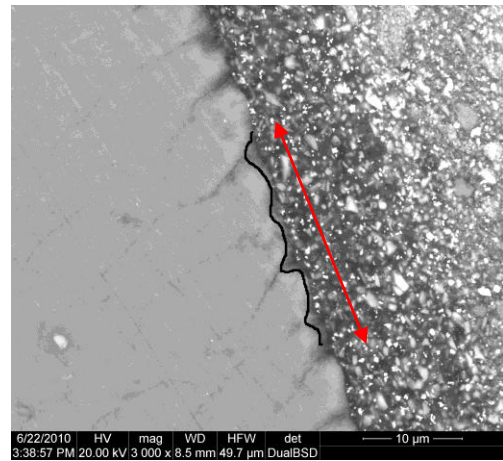
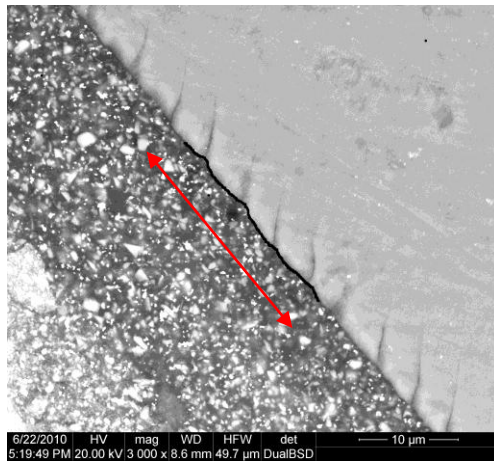


Figure 9: Measuring surface roughness in a cross-sectional view of dental restoration. Red arrow has a 20µm length where the surface roughness of the adhesive is traced in black.

DATA AND STATISTICAL ANALYSIS

Data were analyzed using statistical analysis software, SAS (version 9.1, SAS Institute, Cary, NC). Response variables (bond strength, failure mode, and % resin) were checked for normality and equal variance and were found to have fit the assumptions of linear regression.

The objective was to determine the influence of treatment, scrubbing and non-scrubbing of etchant, on bond strength. Each tooth was cut in half and since each specimen was treated the same, the results were averaged for each tooth in a cluster effect. This way, the variation due to the predictor variable within each tooth can be accounted. Thus, the data is considered repeated because multiple responses were measured from the same tooth. Given the design of the project, the most appropriate model to use was the mixed effect model. Repeated measures and compound symmetry matrix were used to analyze the repeated measures data. Further analysis of data was done to thoroughly evaluate all data. Correlation between response variable and all predictors between treatment groups as well as the amount of total resin to bond strength were evaluated with Pearson's correlation test. Chi square test was used to test the frequency of failure mode in shear bond strength testing. The significance of etch pattern surface roughness was evaluated with paired t-tests. Adjust p-values <0.05 were considered statistically significant.

MICROSHEAR BOND STRENGTH

The simple statistics for the two treatment types, scrubbing and passive application of etchant, is summarized in Tables 1 and 2. A complete dataset can be located in the appendix. The two treatments, scrubbing and passive application are the predictor variables, where response variables are observed as bond strength (Kg and MPa), percent of resin (adhesive or bonding), and failure modes. The mean shear bond strength for the two predictor variables, scrubbing and passive application are 23.56 ± 6.06 and 23.22 ± 5.89 MPa, respectively. Histograms evaluating bond strength values in MPa for each treatment group shows a normal distribution (Figure 11). Normal probability plots also show a normal distribution among treatment groups in MPa (Figure 12). The boxplot shows similar bond strength results for both treatment groups with an evenly distributed range of values (Figure 13). From our results we did not have any obvious outliers and included all data.

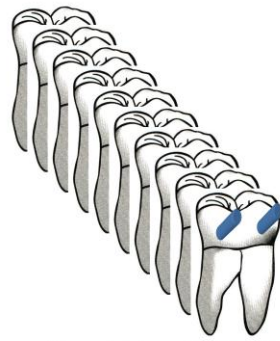
A mixed effect model analysis was performed on the data between the two treatment groups, scrubbing and passive application, and demonstrated no statistical significance when taking in consideration the bond strengths or adhesive resin percentage (p-value >0.05)(Tables 3 and 4).

Table 1: Standard simple statistics for full data set for active “scrubbing” treatment. Column depicting “missing” constitute to a cohesive in tooth (Co-T) failure where further bond strength testing could not be measured.

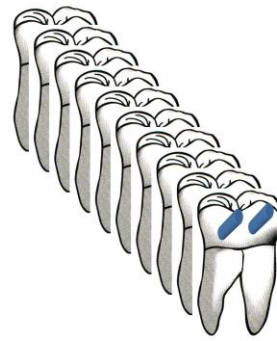
	<i>n</i>	<i>missing</i>	<i>min</i>	<i>max</i>	<i>Mean</i>	<i>SD</i>
Strength (Kg)	38	0	1.12	3.69	2.41	0.62
Strength (MPa)	38	0	10.85	36.12	23.56	6.06
Adh. Resin (%)	37	1	0.94	38.01	10.89	9.49
Bond Resin (%)	17	1	2.31	75.94	21.40	19.61
Total Resin (%)	37	1	2.23	82.21	19.33	17.97
Etch pattern 3,000x	5	0	27.83	35.56	30.90	2.84
Etch pattern 5,000x	5	0	28.29	33.99	30.73	2.38

Table 2: Standard simple statistics for full data set for the control group: “passive” application of etchant.

	<i>n</i>	<i>missing</i>	<i>min</i>	<i>max</i>	<i>Mean</i>	<i>SD</i>
Strength (Kg)	42	0	0.91	3.85	2.38	0.61
Strength (MPa)	42	0	8.86	37.64	23.22	5.89
Adh. Resin (%)	42	0	2.39	37.21	13.06	8.58
Bond Resin (%)	16	0	1.09	34.09	10.56	8.74
Total Resin (%)	42	0	2.48	51.04	15.43	12.55
Etch pattern 3,000x	5	0	20	22.29	21.42	0.73
Etch pattern 5,000x	5	0	20.93	22.22	21.61	0.92



Scrubbing: 10 teeth (38 specimen)



Passive: 10 teeth (42 specimen)

Figure 10: Illustration of sample size for scrubbing and passive treatment group for the microshear bond strength test. Sample size of $n=10$ per treatment group where the composite sticks (blue buttons) vary between 2-3 specimens per tooth. Scrubbing group has a total of 38 specimens and passive group has 42 specimens. The bond strength of specimens on a particular tooth is average due to clustering effect of having the same tooth substrate.

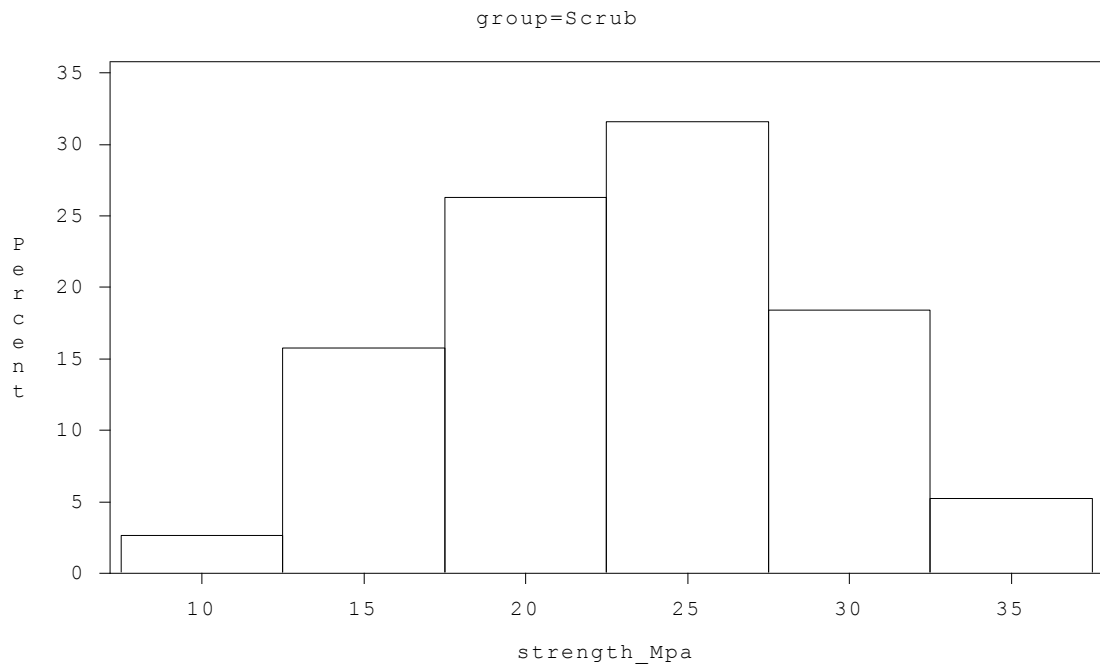
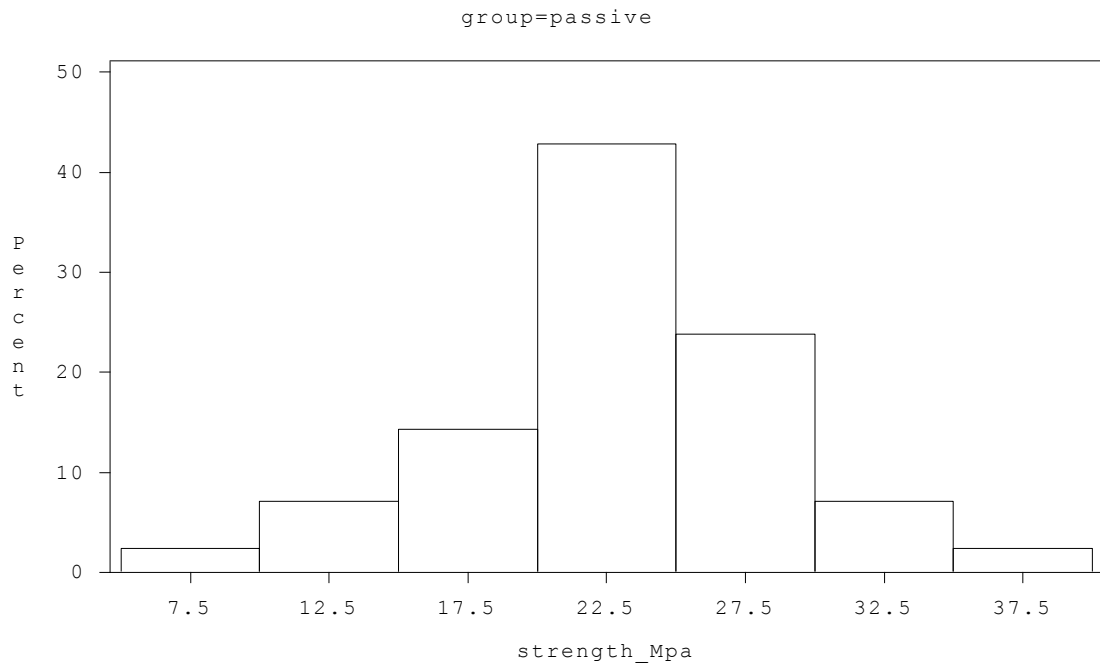


Figure 11: Histogram showing normal distribution of scrubbing and passive application of etchant for microshear bond strength test for (MPa).

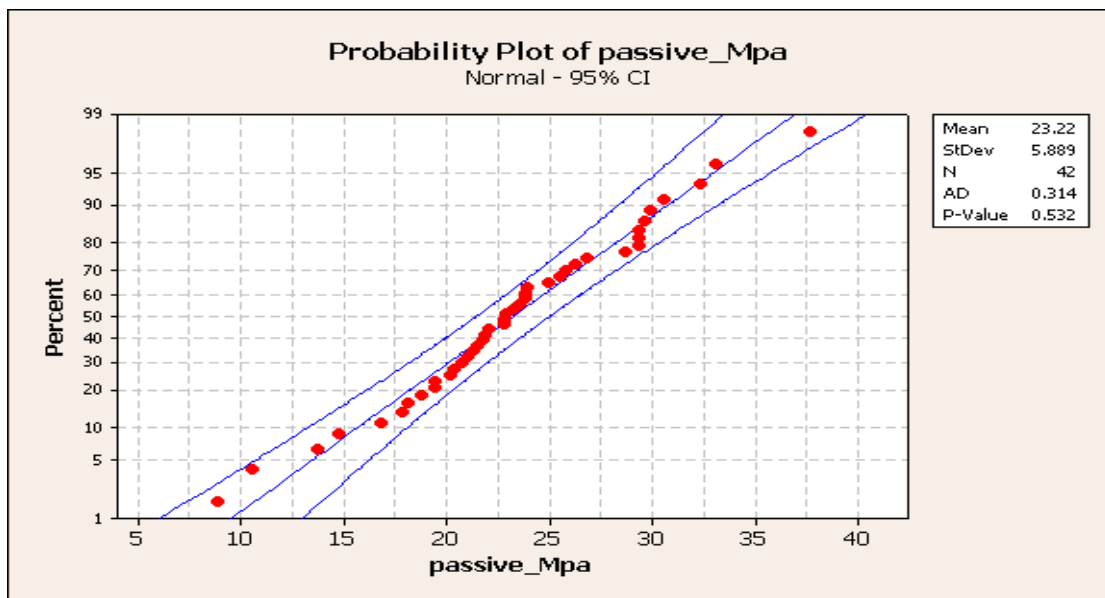
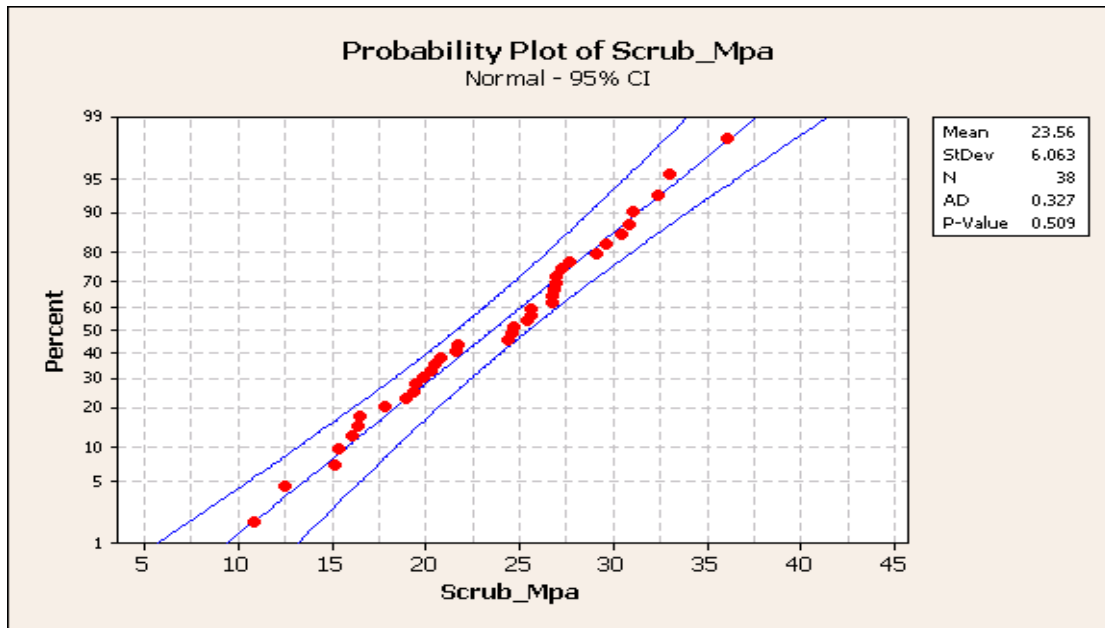


Figure 12: Q-Q plots of the bond strength (MPa) for scrubbing and passive application of etchant groups ($p\text{-value} > 0.05$). For a normal distribution the shape of the curve will follow a straight line where $y=x$. Further analysis for normality was done with Shapiro-Wilks tests with a $p\text{-value}$ of 0.754 for scrubbing group and 0.832 for passive group.

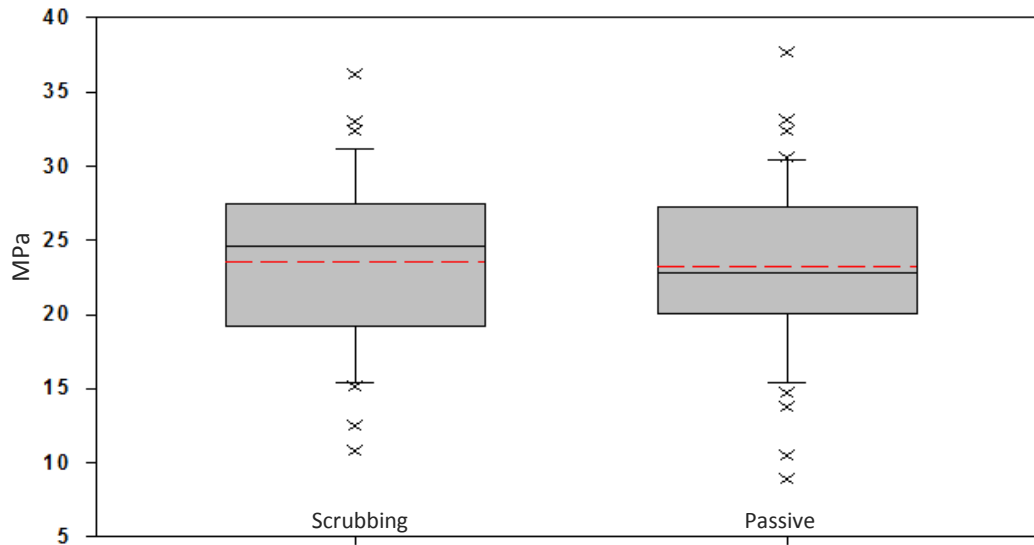


Figure 13: Boxplot of data demonstrating the distribution among individual samples per treatment group for microshear bond strength test. Mean is represented by red dotted lines and is essentially between the 5 and 95% confidence intervals which describe a good distribution.

Table 3: Mixed effect model summery statistics. No significant difference between the two treatments, scrubbing and passive, with regards the microshear bond strength (p-value>0.05).

	<i>Fixed Effects</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>t-value</i>	<i>Pr> t </i>
Strength (Kg)	(Intercept)	2.380	0.095	25.05	<.0001
	Scrubbing	0.029	0.138	0.22	0.829
Strength (MPa)	(Intercept)	23.220	0.922	25.20	<.0001
	Scrubbing	0.340	1.337	0.25	0.799

Correlation is measured to evaluate the statistical strength between two common and continuous variables. Total resin amount of the site of failure was analyzed to the bond strength for any correlation. Both the scrubbing group and passive groups do not

show correlation between total adhesive resin and bond strengths (p-value >0.05). The bond strengths, Kg and MPa are interchangeable, thus, its correlation is disregarded.

Table 4: Correlation matrix of scrubbing application to bond strength.

Pearson Correlation Coefficients, N = 38 Prob > r under H0: Rho=0			
	Strength (Kg)	Strength (MPa)	Total Resin %
Strength (Kg)	1.00000	1.00000 <.0001	0.01937 0.9081
Strength (MPa)	1.00000 <.0001	1.00000	0.01935 0.9082
Total Resin %	0.01937 0.9081	0.01935 0.9082	1.00000

Table 5: Correlation matrix of passive application to bond strength.

Pearson Correlation Coefficients, N = 42 Prob > r under H0: Rho=0			
	Strength (Kg)	Strength (MPa)	Total Resin %
Strength (Kg)	1.00000	0.99876 <.0001	-0.06349 0.6896
Strength (MPa)	0.99876 <.0001	1.00000	-0.06255 0.6939
Total Resin %	-0.06349 0.6896	-0.06255 0.6939	1.00000

SITE OF FAILURE

A graph describing the failure sites in figure 16 represents all specimen failure. Failure was categorized as: i) adhesive interface between tooth and adhesive (A), ii) mixed with tooth and adhesive or composite resin (M), iii) cohesive within tooth (Co-T), or vi) cohesive within the composite (Co-C). Failure were observed either as mixed (M) or adhesive (A) with the exception one which failed cohesively within tooth structure (Co-T) and the data is omitted as an outlier for the statistical purposes when analyzing failure modes.

For both scrubbing and passive treatments, the majority of failure was mixed (M) at 88.61% and the remainder 11.39% were adhesive (A) failures. The type of failure among the two treatment groups were significantly similar where scrubbing group had 10.81% adhesive and 89.19% mixed failure and passive group had 11.90% adhesive and 88.10% mixed failure.

A binary analysis organized the site of failure for the two predictor variable, scrubbing and passive application (Table 4). Only mixed (M) and adhesive (A) failures were considered in the binary analysis.

A chi-square test was performed on the failure site data. The p-value of 0.879 indicated that there was no statistically significant difference in the failure site between the two treatment groups.

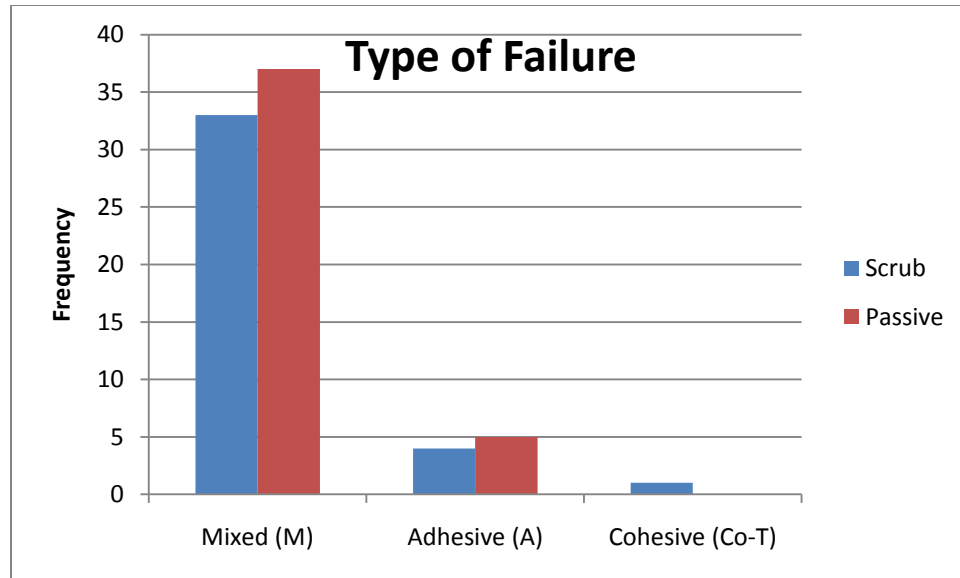


Figure 14: Frequency of the site of failure for scrubbing and passive application of etchant.

Table 6: Frequency of site of failure testing the association between failure modes between treatment types. The binary assessment evaluating the failure modes are mixed (M) and adhesive (A) failure, where the one specimen that failed cohesively within tooth (Co-T) was omitted in the Chi-square analysis. (p-value =0.879 with 1 degree of freedom).

<i>Response Variable</i>		<i>Mixed</i>	<i>Adhesive</i>
Scrubbing	Frequency	33	4
	Percent	89.19	10.81
Passive	Frequency	37	5
	Percent	88.10	11.90

SURFACE ROUGHNESS

Table 7: T-test analysis of surface roughness. Evaluation were done for the 3,000 and 5,000x SEM images and compared the differences of surface roughness between the treatment groups (p-value<0.05).

<i>Difference</i>	<i>n</i>	<i>min</i>	<i>max</i>	<i>Mean</i>	<i>SD</i>	<i>Pr> t </i>
Etch pattern 3,000x	10	5.54	14.53	9.48	2.655	0.0032
Etch pattern 5,000x	10	6.59	13.06	9.124	3.349	0.0015

Table 8: Paired t-tests comparing the differences between the repeated measurements of surface roughness. Differences among treatment groups for the 3,000x and 5,000x SEM images were evaluated since surface roughness per sample was measured twice using the 3,000 and 5,000x images (p-value>0.05).

<i>Difference</i>	<i>n</i>	<i>min</i>	<i>max</i>	<i>Mean</i>	<i>SD</i>	<i>Pr> t </i>
Scrubbing	10	27.83	35.56	33.68	2.619	0.919
Passive	10	20	22.29	22.18	0.729	0.703

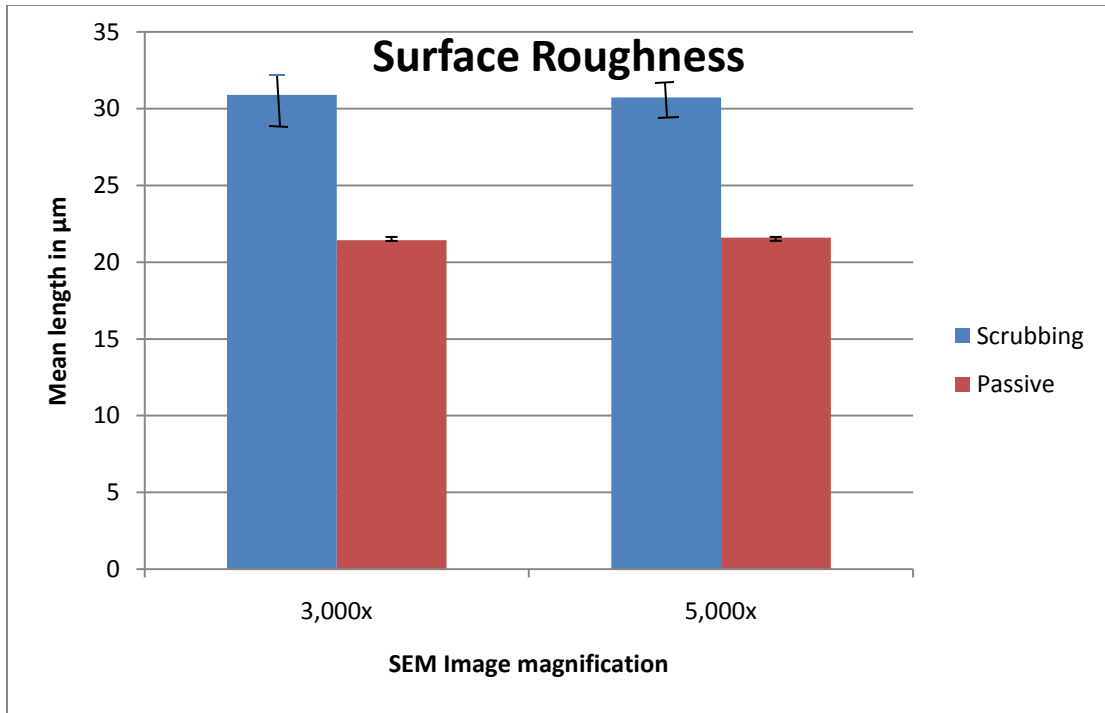


Figure 15: Surface roughness bar graph of the scrubbing and passive treatment groups. Mean lengths of surface roughness measured between a set $20\mu\text{m}$ for SEM images at 3,000 and 5,000x.

The primary functions of etchants are to create a better etch pattern of microporosities, enhanced enamel rods and prisms, greater surface area for bonding, increased bond strength and longevity, and thereby to achieve maximum bonding of tooth to restoration. The objectives of this study were to determine if active scrubbing application of 4th generation phosphoric acid etchant will achieve better and more reliable dental restorations. Microshear bond strength test measured the bond strength between the two methods and failure sites were evaluated. Further analysis of the active etchant treatment was done through SEM imaging. Enamel topography was observed (Figure 7) and etch pattern difference (Figure 8) was quantified by measuring the surface roughness.

The microshear bond strength of resin composite to etched enamel from this study is comparable to other in vitro studies that have an average microshear bond strength of 20MPa (Eick, Robinson, Chappell, Cobb, & Spencer, 1993) (Gwinnett & Kanca, 1992) (Gilpatrick, Ross, & Simonsen, 1991). Observed shear bond strengths in this study for scrubbing and passive application are 23.56 ± 6.06 and 23.22 ± 5.89 MPa, respectively. Thus, the bond strengths in this study are comparable to the bond strengths in literature and imply that bond strengths observed would be clinically significant.

All data measured were included except where the failure was Co-T and bond strength values could not be recorded. The mixed effect model was the most effective test to analyze the microshear bond strength data due to the clustering effect of the specimens per tooth (Figure10). There is no significant difference between the

microshear bond strength values for active and passive application of etchant (Table 3). Simple statistics for the microshear bond strength values show a normal distribution and are illustrated via the histogram, boxplots, and Q-Q plots (Figures 11-13). Normality plot describes a normal distribution with a 95% confidence interval and shows a graph that follows a straight line where $y=x$. According to the Shapiro-Wilks normality test the bond strength value data for scrubbing and passive application of etchant has a p -value >0.05 , thus, the null hypothesis, H_0 , is accepted and states that there is no difference in bond strength between the two treatment groups.

The location where the composite sticks were removed during microshear bond strength test is considered the failure site. The mode of failure at the failure sites was predominantly mixed (88.61%) with occasional failures observed as adhesive (11.41%). Thus, most of the failure occurred with some mixture of composite still left on the interface. One explanation for the majority of mixed failure is due to a strong bond between tooth and composite where the debonding was also occurring in the composite material. This reflects a very strong bond of adhesive to tooth for both treatment groups where in this study the average microshear bond strength is about 23 MPa as compared to 20 MPa as in other in vitro studies.

The sample for the microshear bond strength testing followed a strict protocol where only one operator did the procedures. All samples were treated exactly the same, especially in regards to etch, drying, irradiation, and storage duration to avoid ambiguity in protocol or tooth desiccation. However, enamel variation from tooth to tooth can produce improper etching in some areas and even though this may have been accounted

for the slight differences in bond strength, it is not a factor in the resultant bond strength for values in the study are statistically comparable to published results.

As for failure site analysis, examples in the Figure 6b, typical failures show mixed failure where the composite material still adhered to the tooth structure after shear bond testing. This is probably due to the type of testing where the shear pulling comprises of both tensile and compressive forces. A shear force from the testing machine involves both tensile and compressive forces acting on the interface where the composite specimen meets the tooth surface. Thus, the compression force exerting towards the direction of the tooth during shear bond testing may be the reason as to why there is composite residue adhering on one side of the failure site.

Another conclusion for the majority of mixed failure is that the maximum microshear bond strength has been reached and failure in dental material was the consequence. If most failure includes restoration material of cohesive within composite (Co-C) rather than at the bonding interface, adhesive (A), then perhaps bond strength cannot be measured since breakage is happening in the material rather than tooth-adhesive interface. Therefore, mixed failure with cohesive within composite indicates that the restorative material could not withstand the microshear bond test whereas a majority of adhesive failures would indicate problems with the adhesive material or process of bonding, for example the etch technique. Hence, the differences in treatment of etchant may not be revealed by measuring the microshear bond strength. Trends between the adhesive or composite resin on the failure site and microshear bond strength have been analyzed with Pearson's correlation matrix plot (Figure 14). This analysis was done to see if increase in bond strength may correlate with an increase in resin residing on the

failure site since mixed or cohesive failures are usually associated with higher bond strength. Although there are no significant trends between the two variables, total resin on failure site to bond strength, this is probably due to having reached the optimum bond strength where differences between treatment groups are not shown with microshear bond strength testing.

This study tested scrubbing method of etchant because it has been proposed that active scrubbing of etchant could create better etch patterns by having tooth structure constantly covered with fresh etchant to amplify active chemical interaction of etchant to tooth structure. Also, churning of fresh etchant could prevent air bubbles from forming as well as spread etchant throughout tooth surface. Although the bond strength values for the two groups are similar, other inferences have been made to further analyze the treatment differences in the project. Observation of etched enamel (Figure 7) and analysis of resin infiltration based on surface roughness (Figure 9) have been conducted.

Topographical observation of etched enamel pattern illustrate noticeable differences in etch surface (Figure 7 and appendix B). Treatment of scrubbing and passive application of etchant revealed different surface morphology. These differences were apparent in all five tooth samples and had specific characteristic in etch patterns for each treatment group. Phosphoric acid is known to cause selective dissolution around enamel prism cores and boundaries and creates microporosities ranging from 5-50 μ m deep (Cehreli, 2006). Additional scrubbing application of phosphoric acid revealed increase roughness and more pronounced enamel rods and prisms. Deep and uniform demineralization areas around enamel prism cores were apparent when etchant was scrubbed. Etch patterns from passive application of etchant were subtle when compared

to the scrubbing group. Scrubbing of etchant produces a rougher etch pattern whereas passive application of etchant shows ill-defined surface structures. Thus, a better etch pattern is demonstrated by rougher surface and deeper microporosities providing a larger surface area for increase bonding of adhesive to tooth structure.

Quantitative measurements of surface roughness was done to record the differences in etch patterns between treatment groups (Figure 9 and Appendix C). A standard 20 μ m length was implemented for both the scrubbing and passive groups at 3,000x and 5,000x magnifications. Measurement for one specimen was done twice at 3,000x and 5,000x, and this data was compared using paired t-tests which indicated no significant differences between these repeated measurements. When comparing the surface morphology between the treatment groups, obvious differences in etch pattern was seen. Scrubbing of etchant created a rougher, more irregular surface whereas passively applying etchant did not create deep grooves and peaks. Within a 20 μ m linear length, the average traced length of etched enamel surface is 33.68 ± 2.619 for the scrubbing group and 22.18 ± 0.729 for passive group. Obvious differences between the two groups were analyzed with t-test and gave p-values of 0.0032 and 0.0015 for images at 3,000x and 5,000x, respectively. Thus, there is a significant difference in surface roughness when actively or passively applying acid etchant.

Assessing the surface morphology and surface roughness confirms that there is a difference when actively scrubbing on acid etchant onto enamel. The width of enamel prism cores created with scrubbing are approximately 5 μ m, as seen on surface topography images. Cross-section of dental restoration showed images of resin infiltration, displaying a saw-tooth like etch surface where the distance between the peaks

are also approximately 5µm across. These two procedures verify that the etch surface caused by scrubbing phosphoric acid creates more obvious demineralization around enamel prism cores and boundaries since the prism cores of approximately 5µm can be seen by two different views (Figures 7 and 9).

Scrubbing of etchant onto enamel creates surface irregularities and microporosities that can be easily filled with adhesives creating better bonding. A more pronounced etch pattern allows the inflow of adhesive and bonding agent into the porous zone and will ultimately form resin tags and a more intimate micromechanical retention to etched enamel. It has been reported that a highly secured bond could decrease micro and nanoleakage which prevents future bacterial infection or early deterioration of restoration. Microshear bond strength does not show significant differences between the two treatments, indicating that maximum bond strength is achieved regardless of scrubbing or passive application methods. However, a better etch pattern may allow better bonding where a more secure bond could ultimately increase longevity by withstanding leakage, thermo differences, and wear of restoration.

CHAPTER VII: CONCLUSION

The purpose of this study was to examine the effects of actively scrubbing on phosphoric acid of 4th generation etch-and-rinse adhesives systems as compared to passive application of etchant. From the results of this investigation, it can be concluded: (1) The microshear bond strength values between scrubbing and passive application of acid etchant are significantly different. Microshear bond strength values are comparable to published data and techniques are applicable to clinical settings. (2) Failure sites were in general, mostly mixed with tooth and composite. High bond strengths and a majority of mixed failure suggest maximum bond strength have been reached. (3) Etched enamel surface demonstrate obvious differences in enamel morphology between the two treatments. (4) Quantitative analysis of surface roughness between the two treatments are significantly different. Active scrubbing of acid etchant creates more irregular etch pattern with more pronounced enamel prisms and rods and deeper microporosities. Thus, scrubbing of acid etchant onto enamel may increase dental restoration longevity and durability by creating increased surface area for a more secure and better bonding of restoration material to tooth structure.

REFERENCES

- Ando, S., T. Watanabe, et al. (2008). "Effect of adhesive application methods on bond strength to bovine enamel." J Oral Sci: 50(52):181-186.
- Bastos, P., D. Retief, et al. (1988). "Effect of etch duration on the shear bond strength of microfilm composite resin to enamel." Am J Dent 1: 151-157.
- Bates, D., D. Retief, et al. (1982). "Effects of acid etch parameters on enamel topography and composite resin-enamel bond strength. ." Pediatr Dent 4: 106-110.
- Bayne, S., J. Flemming, et al. (1992). "SEM-EDS analysis of macro and micro resin tags of laminates." J Dent Res 61: 304.
- Bertolotti, R. L. (1991). "Total etch--the rational dentin bonding protocol." J Esthet Dent 3(1): 1-6.
- Black, G. V. (1917). "A Work on Operation Dentistry in Two Volumes." Chicago: Medico-Dental Publishing 3rd edition.
- Blosser, R. (1990). "Time dependence of 2.5% nitric acid solution as an etchant on human dentin and enamel." Dent Mater 6: 83-87.
- Bolanos-Carmona, V., S. Gonzalez-Lopez, et al. (2006). "Effects of etching time of primary dentin on interface morphology and microtensile bond strength." Dent Mater: 12(12):1121-1129.
- Bolanos-Carmona, V., S. Gonzalez-Lopez, et al. (2008). "Interfacial morphology and bond strength of self-etching adhesives to primary dentin with or without acid etching." J Biomed Mater Res B Appl Biomater: 87(82): 499-507.
- Bowen, R. L., Eick, J.D., Henderson, D.A., et al (1984). "Smear layer: removal and bonding considerations." Oper Dent Suppl 3: 30-34.
- Brown, L. J., D. M. Winn, et al. (1996). "Dental caries, restoration and tooth conditions in U.S. adults, 1988-1991. Selected findings from the Third National Health and Nutrition Examination Survey." J Am Dent Assoc 127(9): 1315-1325.
- BT, R., & J, P. (2000). Bond strengths of nonrinsing adhesives. Quintessence Int , 31:355-358.

Buonocore, M. G. (1955). "A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces." J Dent Res 34(6): 849-853.

Cehreli, S. and N. Eminkahyagil (2006). "Effect of active pretreatment of self-etching primers on the ultramorphology of intact primary and permanent tooth enamel." J Dent for Children: 73(72):86-90.

Chan, K., F. Tay, et al. (2003). "bonding of mild self-etching primers/adhesives to dentin with thick smear layers." Am J Dent: 12:340-346.

Dal-Biano, K., A. Pellizzaro, et al. (2006). "Effects of moisture degree and rubbing action on the immediate resin-dentin bond strength." Dent Mater: 22(12):1150-1156.

De Munck, J., B. Van Meerbeek, et al. (2003). "Microtensile bond strengths of one- and two-step self-etch adhesives to bur-cut enamel and dentin." Am J Dent: 16: 414-420.

do Amaral, R., R. Stanislawczuk, et al. (2009). "Active application improves the bonding performance of self-etch adhesives to dentin." J Dent: 82-90.

Duke, E. S. (1993). "Adhesion and its application with restorative materials." Dent Clin North Am 37: 329-340.

Eakle, W. S. (1986). "Fracture resistance of teeth restored with class II bonded composite resin." J Dent Res 65(2): 149-153.

Eick, J. D., C. M. Cobb, et al. (1991). "The dentinal surface: its influence on dentinal adhesion. Part I." Quintessence Int 22(12): 967-977.

Eick, J., Robinson, S., Chappell, P., Cobb, C., & Spencer, P. (1993). The dentinal surface: Its influence on dentinal adhesion. Part III. Quintessence Int , 24:571-582.

Eick, J. D., S. J. Robinson, et al. (1993). "Adhesives and nonshrinking dental resins of the future." Quintessence Int 24(9): 632-640.

Erickson, R. (1992). "Surface interactions of dentin adhesive materials." Oper Dent suppl 5: 81-91.

Finger, W. J. and S. Uno (1996). "Bond strength of Gluma CPS using the moist dentin bonding technique." Am J Dent 9(1): 27-30.

Fusayama, T., M. Nakamura, et al. (1979). "Non-pressure adhesion of a new adhesive restorative resin." J Dent Res 58(4): 1364-1370.

Gaengler, P., I. Hoyer, et al. (2004). "Micromorphological evaluation of posterior composite restorations - a 10-year report." J Oral Rehabil 31(10): 991-1000.

Gilpatrick, R., Ross, J., & Simonsen, R. (1991). Resin-to-enamel bond strengths with various etching times. Quintessence Int, 22:47-49.

Gwinnett, A. J. (1990). "Interactions of dental materials with enamel." Trans Acad Dent Mater 3: 30.

Gwinnett, A., & Kanca, J. (1992). Micromorphology of the bonded dentin interface and its relationship to bond strength. Am J Dent, 5:73-77.

Heymann, H. O. B., S.C. (1993). "Current concepts in dentin bonding: Focusing on dentinal adhesion factors." J Am Dent Assoc 124: 27-36.

Higashi, C., M. D. Michel, et al. (2009). "Impact of adhesive application and moisture on the mechanical properties of the adhesive interface determined by the nano-indentation technique." Oper Dent 34(1): 51-57.

Holtan, J., G. Nystrom, et al. (1995). "Influence of different etchants and etching times on shear bond strength." Oper Dent: 20(23):94-29.

Inoue, I., M. Vargas, et al. (2003). "Microtensile bond strength of eleven contemporary adhesives to enamel." Am J Dent 16: 329-334.

Inoue, S., M. Vargas, et al. (2001). "Microtensile bond strength of eleven contemporary adhesives to dentin." J Adhes Dent 3: 237-245.

Jacobsen, T. and K. J. Soderholm (1998). "Effect of primer solvent, primer agitation, and dentin dryness on shear bond strength to dentin." Am J Dent 11(5): 225-228.

Jendresen, M. D., P. O. Glantz, et al. (1981). "Microtopography and clinical adhesiveness of an acid etched tooth surface. An in-vivo study." Acta Odontol Scand 39(1): 47-53.

Kanca, J., 3rd (1992). "Effect of resin primer solvents and surface wetness on resin composite bond strength to dentin." Am J Dent 5(4): 213-215.

Kerdvongbundit, V., S. Thiradilok, et al. (2004). "The use of the replica technique to record fluid emerging from exposed dentine." Arch Oral Biol 49(8): 613-619.

Kidd, E. A. (1976). "Microleakage: a review." J Dent 4(5): 199-206.

- Legler, L., D. Retief, et al. (1990). "Effects of phosphoric acid concentration and etch duration on enamel depth of etch: an in vitro study." Am J Orthod Dentofacial Orthop: 98(92):154-160.
- Miears, J. R., Jr., D. G. Charlton, et al. (1995). "Effect of dentin moisture and storage time on resin bonding." Am J Dent 8(2): 80-82.
- Miyazaki, M., K. Hinoura, et al. (2002). "Effect of self-etching primer application method on enamel bond strength." Am J Dent: 15:412-416.
- Miyazaki, M., J. Platt, et al. (1996). "Influence of dentin primer application methods on dentin bond strengths." Oper Dent: 21(24):167-172.
- Mjor, I. A. (1998). "Amalgam and composite resin restorations: Longevity and reasons for replacement in Anusavice K." Quality Evaluation of Dental Restorations. Quintessence Publishing: 61-80.
- Morin, D., R. DeLong, et al. (1984). "Cusp reinforcement by the acid-etch technique." J Dent Res 63(8): 1075-1078.
- Moura, S., A. Pelizzaro, et al. (2006). "Does the acidity of self-etching primers affect bond strength and surface morphology of enamel?" J Adhes Dent: 8(2):75-83.
- Nordenvall, K.-J., M. Brannstrom, et al. (1980). "Etching of deciduous teeth and young and old permanent teeth. A comparison between 15 and 60 seconds of etching. ." Am J Orthod 78: 99-108.
- Oliveira, S., S. Marshall, et al. (2004). "The effect of a self-etching primer on the continuous demineralization of dentin. ." European J Oral Surgery: 112:376-183.
- Pashley, D. H. (1992). "The effects of acid etching on the pulpodentin complex." Oper Dent 17: 229-242.
- Pashley, D. H., B. Ciucchi, et al. (1993). "Permeability of dentin to adhesive agents." Quintessence Int 24(9): 618-631.
- Perdiago, J. (2007). "New Developments in Dental Adhesion." Dent Clin N Am 51: 333-357.
- Perdiago, J., G. Gomes, et al. (2005). "Enamel bond strengths of pairs of adhesives from the same manufacturer." Oper Dent 30: 492-499.
- Pioch, T., S. Stotz, et al. (1998). "Influence of different etching times on hybrid layer formation and tensile bond strength." Am J Dent: 11:202-206.

Reinhard, H., and Juergen, M. (2001). "Longevity of restorations in posterior teeth and reasons for failure." J Adhes Dent 3: 45-64.

Reis, A., A. Pellazaro, et al. (2007). "Impact of adhesive application to wet and dry dentin on long-term resin-dentin bond strengths." Oper Dent: 32(34):380-387.

Retief, D., H. Busscher, et al. (1986). "A laboratory evaluation of three etching solutions." Dent Mater 2: 202-206.

Rotta, M., P. Bresciani, et al. (2007). "Effects of phosphoric acid pretreatment and substitution of bonding resin on bonding effectiveness" (6) 537-545.

Salz, U., J. Zimmermann, et al. (2005). "Self-curing, self-etching adhesive cement systems." J Adhes Dent 7(1): 7-17.

Sano, H., T. Shono, et al. (1994). "Microporous dentin zone beneath resin-impregnated layer." Oper Dent 19(2): 59-64.

Silverstone, L. M., C. A. Saxton, et al. (1975). "Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy." Caries Res 9(5): 373-387.

S, U., & WJ, F. (1999). Effects of acid conditioner on dentin demineralization and dimension of hybrid layers. *J Dent* , 24:211-214.

Spencer, P., Y. Wang, et al. (2000). "Interfacial chemistry of the dentin/adhesive bond." J Dent Res: 79:1458-1463.

Strassler, H. E. (1991). "Insights and innovations." Dent Off 3: 114.

Swift, E. J. and B. Cloe (1993). "Shear bond strengths of new enamel etchants." Am J Dent: (3) 162-164.

Swift, E. J., Jr., J. Perdigao, et al. (1995). "Bonding to enamel and dentin: a brief history and state of the art, 1995." Quintessence Int 26(2): 95-110.

Swift, E. J., Perdigao, J., Heymann, H.O. (1995). "Bonding to enamel and dentin; a brief story and state of the art." Quintessence Int 26: 95-110.

Tao, L. and D. H. Pashley (1988). "Shear bond strengths to dentin: effects of surface treatments, depth and position." Dent Mater 4(6): 371-378.

Tay, F. and D. Pashley (2001). "Aggressiveness of contemporary self-etching systems.I. Depth of penetration beyond dentin smear layers." Dent Mater: 17:296-308.

Torstenson, B. and A. Oden (1989). "Effects of bonding agent types and incremental techniques on minimizing contraction gaps around resin composites." Dent Mater 5(4): 218-223.

Triolo, P. J., E. J. Swift, et al. (1993). "Effects of etching time on enamel bond strengths." Am J Dent: 6(6):302-304.

Uno, S. and W. Finger (1995). "Effect of acid composition and etch duration on enamel loss and resin composite bonding." Am J Dent: 8(4):164-169.

Van Landyt, K., P. Kanumilli, et al. (2005). "Bond strength of a mild self-etch adhesive with and without prior acid-etching." J Dent: (1) 77-85.

Van Meerbeek, B., J. De Munck, et al. (2003). "Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges." Oper Dent 28: 215-235.

Van Meerbeek, B., P. Kanumilli, et al. (2005). "A randomized controlled study evaluating the effectiveness of a two-step self-etch adhesive with and without selective phosphoric-acid etching of enamel." Dent Mater: (21) 375-383.

Van Meerbeek, B., Vargas, M., Inoue, S., et al (2001). "Adhesives and cements to promote preservation dentistry." Oper Dent Suppl 6: 119-124.

Van Meerbeek, B., Y. Yoshida, et al. (1998). "A TEM study of two water-based adhesive systems bonded to dry and wet dentin." J Dent Res 77(1): 50-59.

Velasquez, L. and R. Sargent (2006). "Effect of placement agitation and place time on the shear bond strength of 3 self-etching adhesives." Oper Dent: 31(34):426-430.

Velasquez, L. M., Sargent, R.S., Burgess, J.O., Mercante, D.E. (2006). "Effect of placement agitation and placement time on the shear bond strength of 3 self-etching adhesives." Oper Dent 31(4): 426-430.

Wang, Y. and P. Spencer (2004). "Effect of acid etching time and technique on interfacial characteristics of the adhesive-dentin bond using differential staining." Eur J Oral Sci: (112) 293-299.

APPENDIX A: STATISTICAL ANALYSIS

Table 7: Raw data from microshear bond strength testing.

<i>group</i>	<i>Tooth</i>	<i>Strength (kg)</i>	<i>Strength (Mpa)</i>	<i>SEM FAILURE</i>	<i>SEM FAIL BINARY</i>	<i>Adh Resin</i>	<i>Bond Resin</i>	<i>Total Resin</i>
Scrub	1.000	1.109	10.848	Mixed	1	2.23		2.23
Scrub	1.000	1.827	17.869	Mixed	1	10.13	3.1	13.23
Scrub	1.000	3.150	30.804	Mixed	1	12.96	4.86	17.82
Scrub	1.000	3.370	32.955	Mixed	1	15.26	2.34	17.6
Scrub	3.000	2.604	25.459			na	na	0
Scrub	5.000	1.649	16.129	Adhesive	2	0		0
Scrub	5.000	1.672	16.351	Adhesive	2	0		0
Scrub	5.000	1.685	16.477	Mixed	1	13.04	2.31	15.35
Scrub	5.000	2.131	20.842	Mixed	1	7.64	43.5	51.14
Scrub	7.000	2.209	21.601	Mixed	1	6.02		6.02
Scrub	7.000	1.989	19.450	Mixed	1	3.62	35.02	38.64
Scrub	7.000	2.222	21.727	Mixed	1	10.33	22.81	33.14
Scrub	7.000	1.572	15.370	Mixed	1	6.96		6.96
Scrub	9.000	1.552	15.181	Mixed	1	1.01	29.28	30.29
Scrub	9.000	2.620	25.617	Mixed	1	5.76	17.66	23.42
Scrub	9.000	2.739	26.787	Mixed	1	32.51	9.76	42.27
Scrub	9.000	2.617	25.586	Mixed	1	23.33	4.35	27.68
Scrub	9.000	1.979	19.355	Mixed	1	0.94	36.65	37.59
Scrub	11.000	1.937	18.944	Mixed	1	3.43	24.86	28.29
Scrub	11.000	2.076	20.304	Mixed	1	3.68		3.68
Scrub	11.000	2.093	20.462	Mixed	1	6.27	75.94	82.21
Scrub	11.000	2.038	19.925	Adhesive	2	0		0
Scrub	13.000	2.516	24.605	Mixed	1	9.72		9.72
Scrub	13.000	1.281	12.524	Mixed	1	18.36		18.36
Scrub	13.000	2.746	26.851	Mixed	1	24.15		24.15
Scrub	13.000	3.027	29.602	Mixed	1	17.87		17.87
Scrub	15.000	2.756	26.946	Mixed	1	6.6	8.02	14.62
Scrub	15.000	2.523	24.669	Mixed	1	2.09	10.63	12.72
Scrub	15.000	2.791	27.293	Mixed	1	11.34		11.34
Scrub	15.000	2.494	24.384	Mixed	1	2.43		2.43
Scrub	17.000	2.756	26.946	Mixed	1	11.01	32.64	43.65
Scrub	17.000	2.733	26.724	Adhesive	2	0		0
Scrub	17.000	3.108	30.393	Mixed	1	38.01		38.01
Scrub	17.000	3.173	31.025	Mixed	1	37.65		37.65

Scrub	19.000	3.312	32.385	Mixed	1	6.74		6.74
Scrub	19.000	3.694	36.117	Mixed	1	6.36		6.36
Scrub	19.000	2.972	29.065	Mixed	1	3.6		3.6
Scrub	19.000	2.833	27.705	Mixed	1	9.89		9.89
passive	2	1.407	13.757	Mixed	1	4.53	12.22	16.75
passive	2	2.180	21.316	Mixed	1	37.21	12.03	49.24
passive	2	2.122	20.747	Mixed	1	4.18	1.09	5.27
passive	2	0.906	8.855	Adhesive	2	0		0
passive	2	2.335	22.834	Mixed	1	11.14	1.13	12.27
passive	4	2.222	21.727	Mixed	1	na	na	0
passive	4	2.380	23.277	Mixed	1	na	na	0
passive	4	2.738	26.787	Mixed	1	10.81		10.81
passive	4	2.196	21.474	Mixed	1	15.74		15.74
passive	6	1.983	19.387	Mixed	1	2.39	16.01	18.4
passive	6	2.439	23.846	Mixed	1	7.83		7.83
passive	6	2.335	22.834	Mixed	1	20.23	3.89	24.12
passive	6	1.507	14.738	Mixed	1	7.21		7.21
passive	8	2.998	29.318	Mixed	1	7.87	1.18	9.05
passive	8	2.238	21.885	Mixed	1	11.17		11.17
passive	8	1.856	18.153	Mixed	1	28.19	14.14	42.33
passive	8	2.406	23.530	Mixed	1	6.71		6.71
passive	10	2.684	26.250	Mixed	1	14.34	5.18	19.52
passive	10	1.077	10.532	Adhesive	2	0		0
passive	10	2.555	24.985	Mixed	1	20.85		20.85
passive	10	2.067	20.209	Mixed	1	27.69		27.69
passive	12	1.717	16.794	Mixed	1	24.78	4.36	29.14
passive	12	2.148	21.000	Mixed	1	4.97	7.65	12.62
passive	12	2.442	23.878	Mixed	1	11.65		11.65
passive	12	1.924	18.818	Mixed	1	10.19	6.91	17.1
passive	14	3.590	33.105	Mixed	1	12.71		12.71
passive	14	3.001	29.349	Mixed	1	22.48		22.48
passive	14	2.613	25.554	Mixed	1	16.32		16.32
passive	14	2.636	25.775	Mixed	1	19.6		19.6
passive	16	1.827	17.869	Mixed	1	13.23		13.23
passive	16	1.989	19.450	Adhesive	2	0		0
passive	16	2.432	23.783	Mixed	1	29.21		29.21
passive	16	2.338	22.866	Mixed	1	24.76		24.76
passive	18	2.083	20.367	Mixed	1	6.02	19.61	25.63
passive	18	3.060	29.918	Mixed	1	3.99	18.37	22.36
passive	18	3.305	32.322	Mixed	1	2.48		2.48

passive	18	2.933	28.685	Mixed	1	11.51		11.51
passive	20	3.027	29.602	Mixed	1	6.82		6.82
passive	20	3.849	37.635	Mixed	1	3.63	11.07	14.7
passive	20	2.258	22.075	Mixed	1	16.95	34.09	51.04
passive	20	3.008	29.381	Adhesive	2	0		0
passive	20	3.124	30.551	Adhesive	2	0		0

Table 8: Raw data for resin infiltration depicting the length of surface roughness over 20µm for active and passive application of etchant at 3,000 and 5,000x.

<i>Treatment</i>	<i>sample</i>	<i>3,000x Length (µm)</i>	<i>5,000x length (µm)</i>
Scrub	A	30.57	28.82
Scrub	B	29.94	28.29
Scrub	C	35.56	33.99
Scrub	D	30.62	32.22
Scrub	E	27.83	30.33
Passive	A	20.0	21.5
Passive	B	21.9	21.7
Passive	C	21.03	20.93
Passive	D	21.9	21.68
Passive	E	22.29	22.22

APPENDIX B: IMAGES OF ETCHED SURFACE

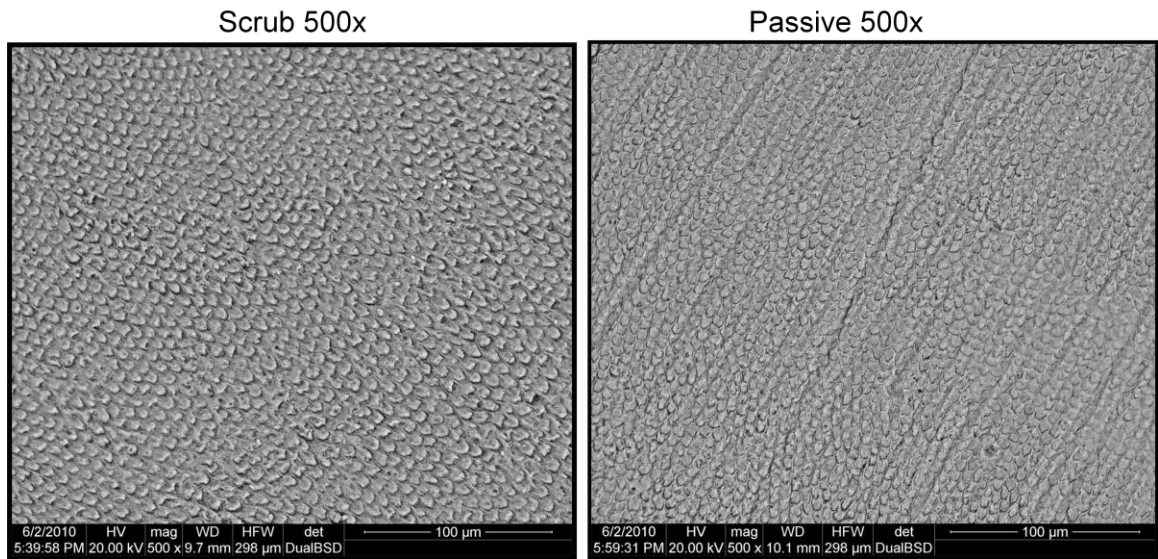


Figure 16: Etch surface morphology of tooth A. Scrubbing is actively applying etchant and passive is passively applying etchant.

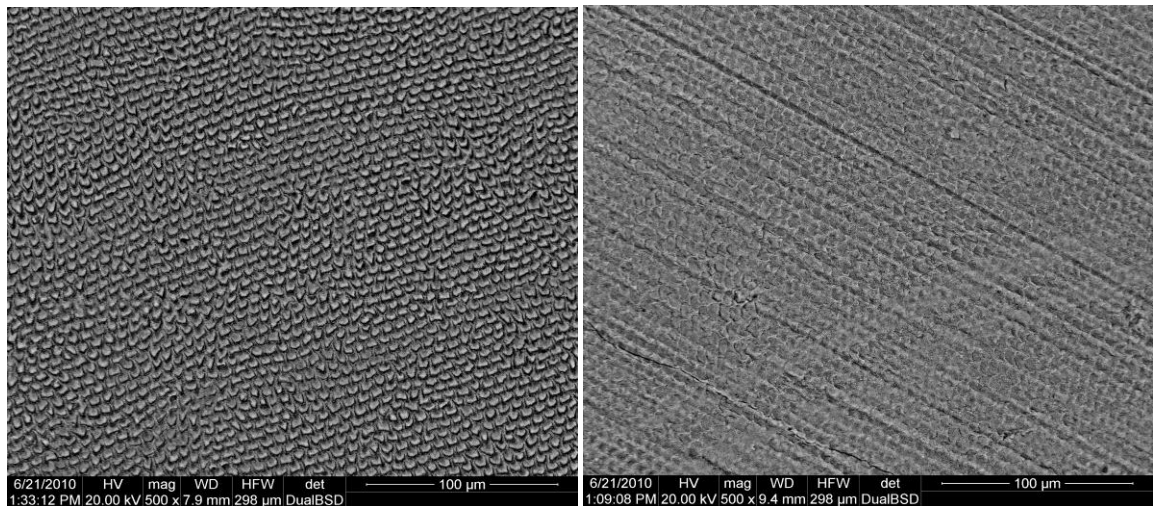


Figure 17: Etch surface morphology of tooth B at 500 x (left is scrub and at right is passive application).

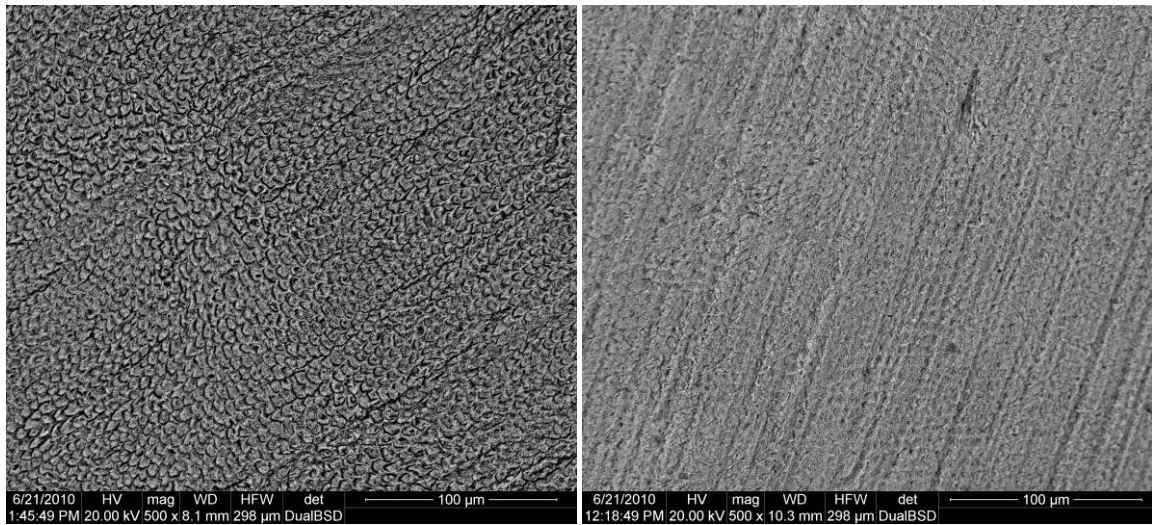


Figure 18: Etch surface morphology of tooth C at 500 x (left is scrub and at right is passive application).

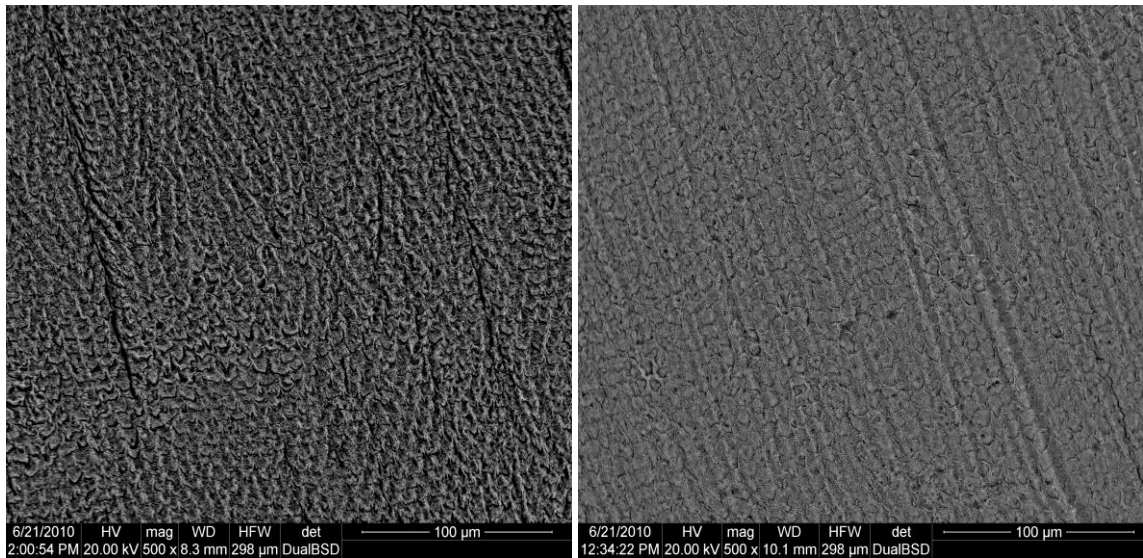


Figure 19: Etch surface morphology of tooth D at 500 x (left is scrub and at right is passive application).

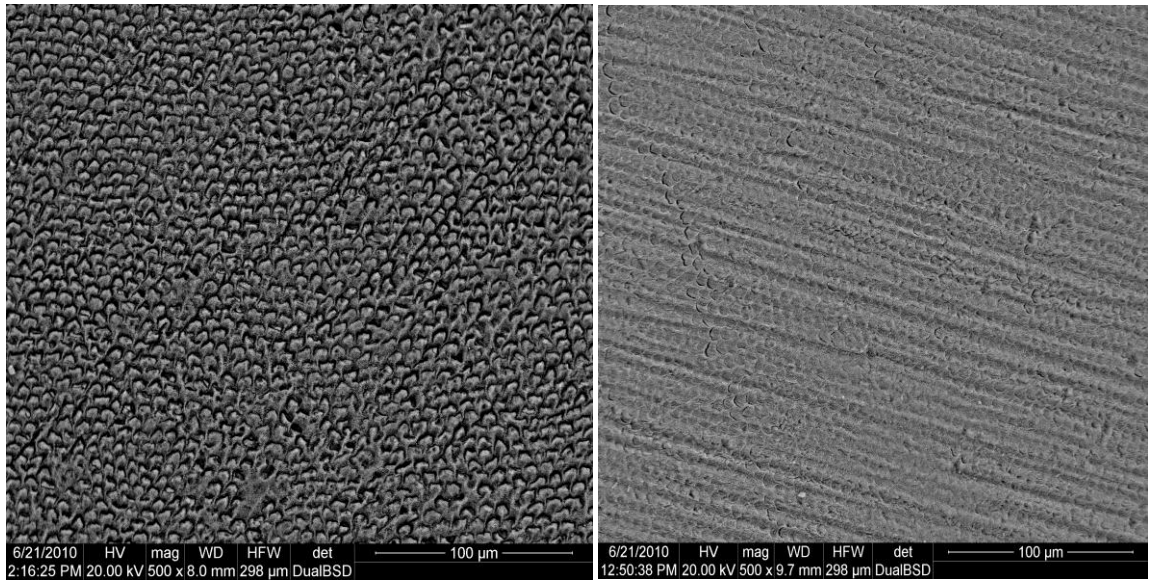


Figure 20: Etch surface morphology of tooth E at 500 x (left is scrub and at right is passive application).

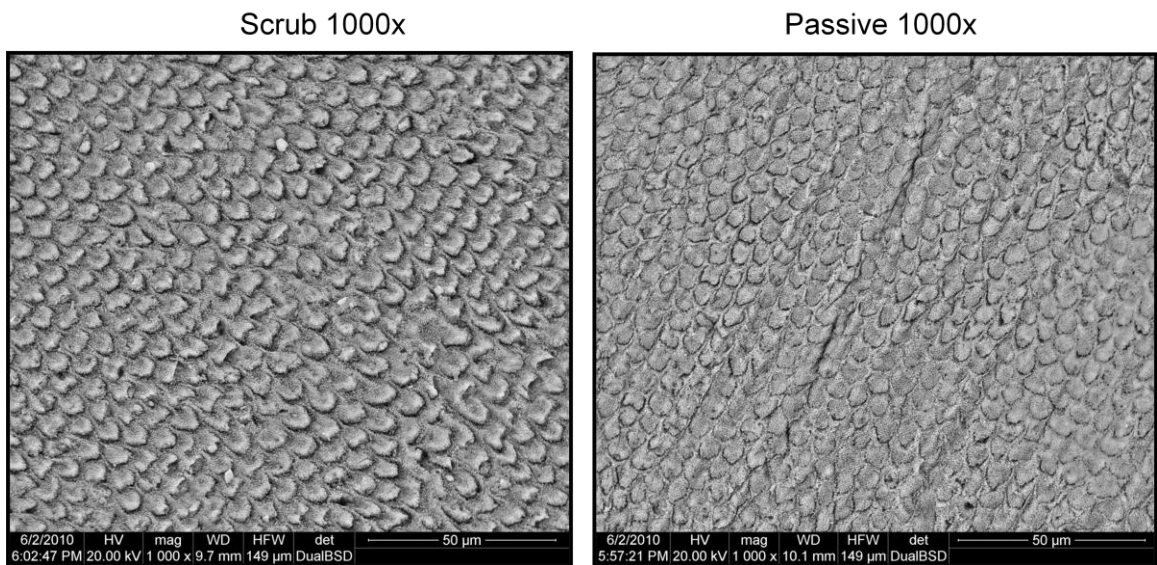


Figure 21: Etch surface morphology of tooth A at 1000 x (left is scrub and at right is passive application).

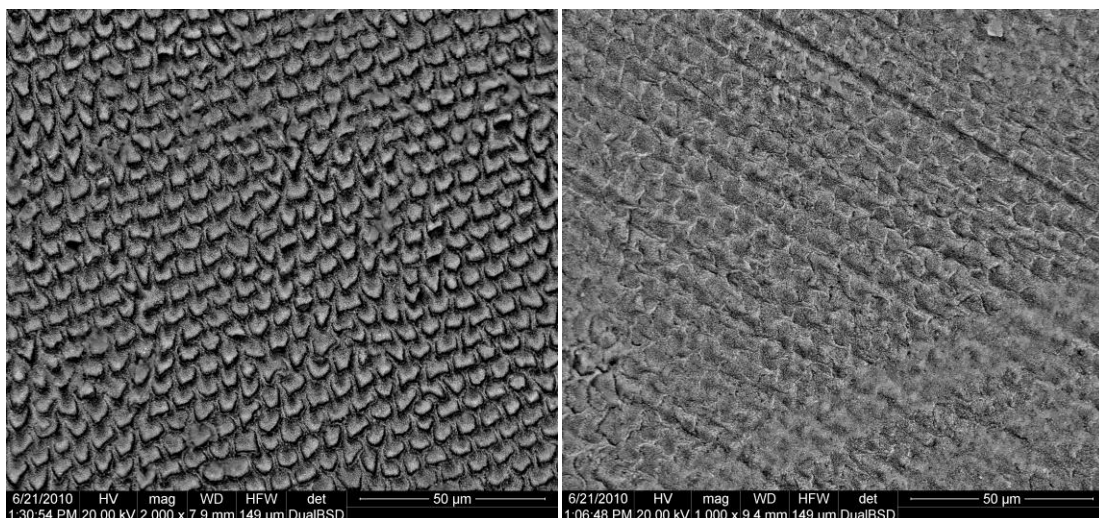


Figure 22: Etch surface morphology of tooth B at 1000 x (left is scrub and at right is passive application).

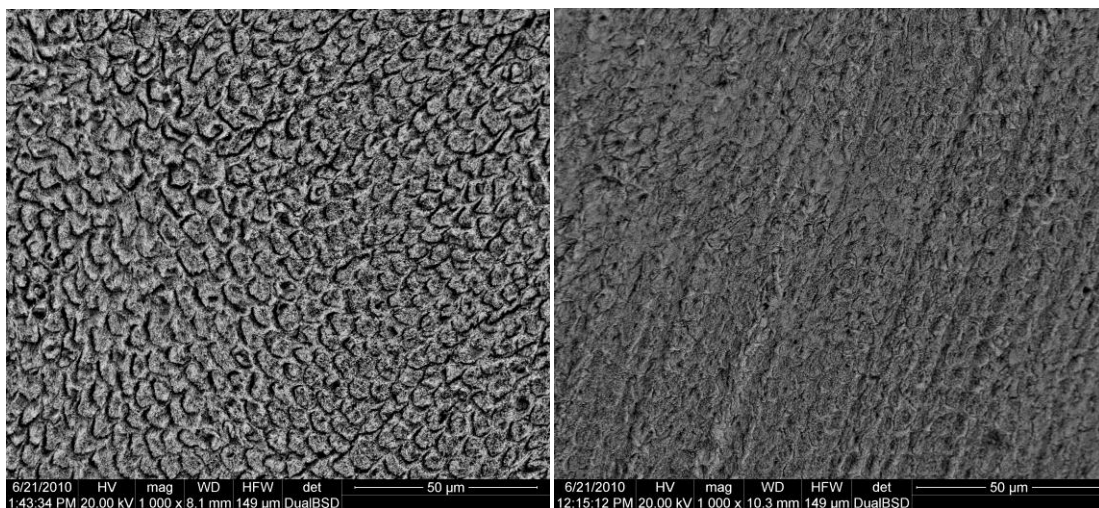


Figure 23: Etch surface morphology of tooth C at 1000 x (left is scrub and at right is passive application).

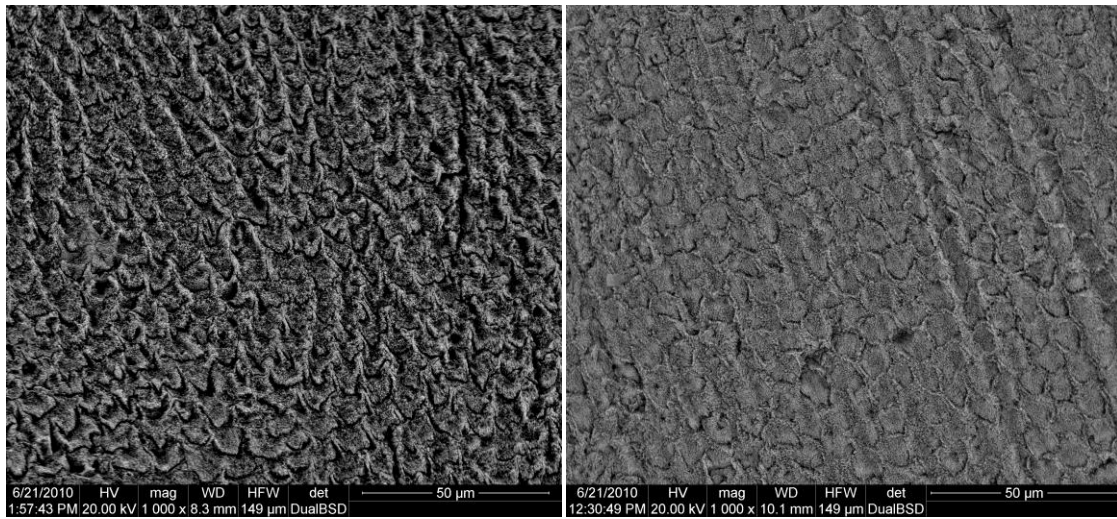


Figure 24: Etch surface morphology of tooth D at 1000 x (left is scrub and at right is passive application).

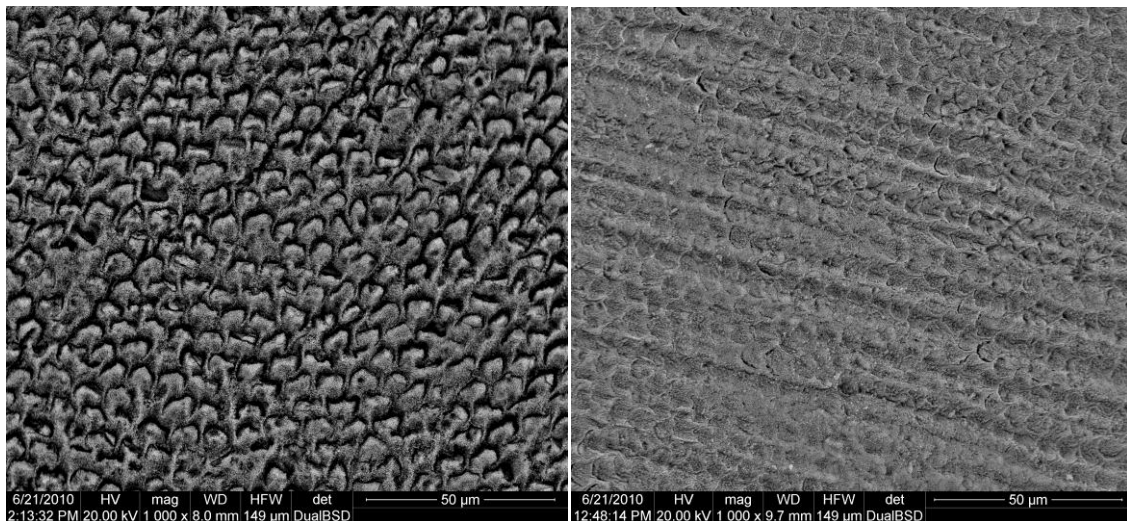
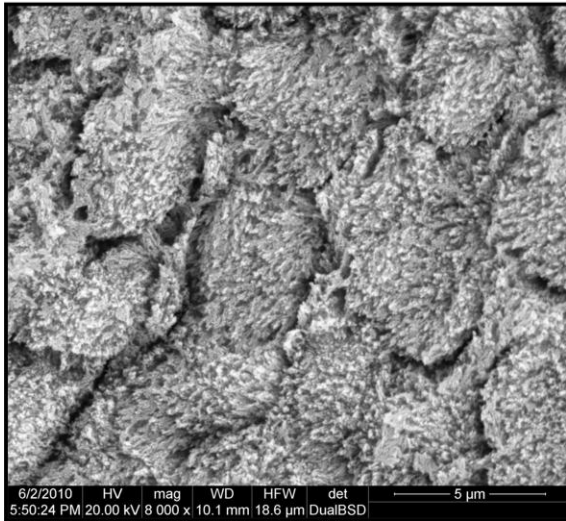


Figure 25: Etch surface morphology of tooth E at 1000 x (left is scrub and at right is passive application).

Scrub 8000x



Passive 8000x

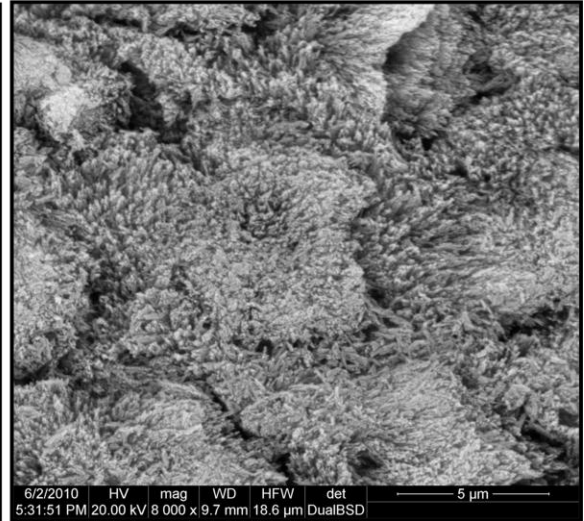


Figure 26: Etch surface morphology of tooth A at 8000 x (left is scrub and at right is passive application).

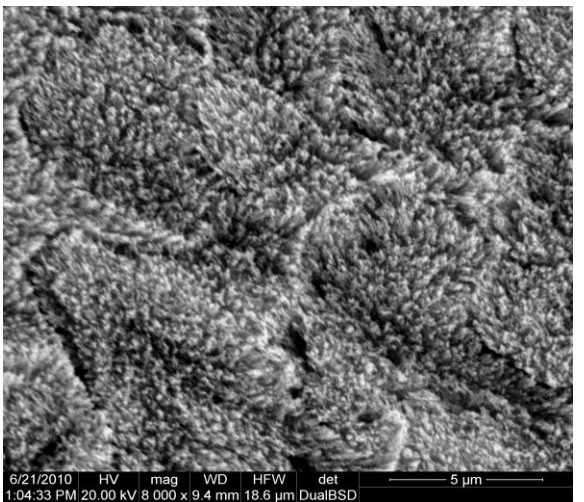
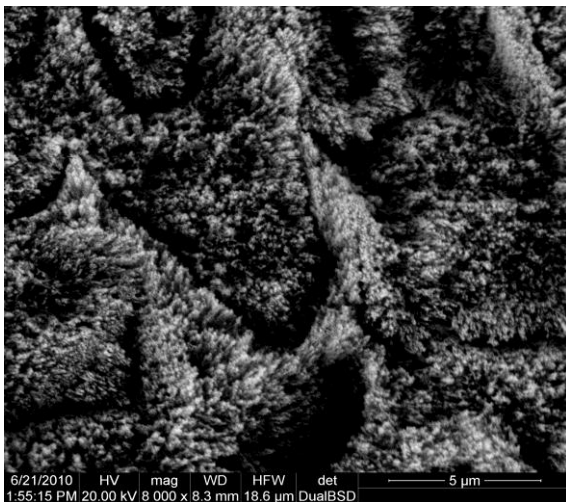


Figure 27: Etch surface morphology of tooth B at 8000 x (left is scrub and at right is passive application).

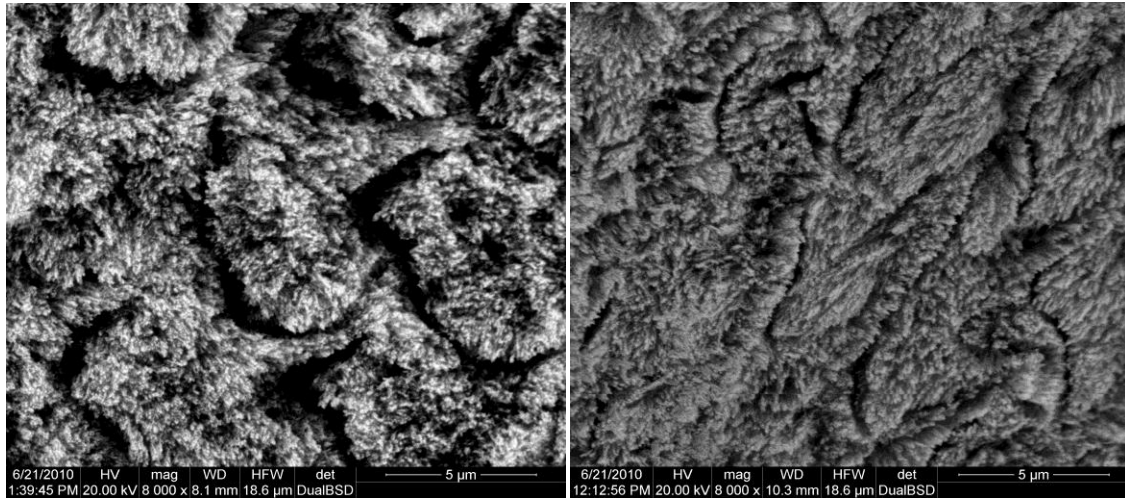


Figure 28: Etch surface morphology of tooth C at 8000 x (left is scrub and at right is passive application).

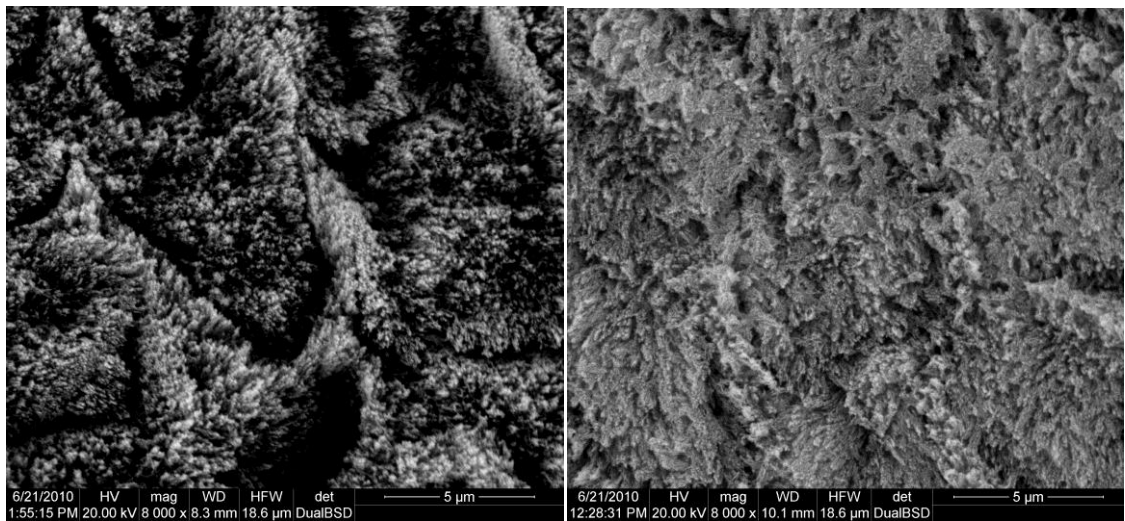


Figure 29: Etch surface morphology of tooth D at 8000 x (left is scrub and at right is passive application).

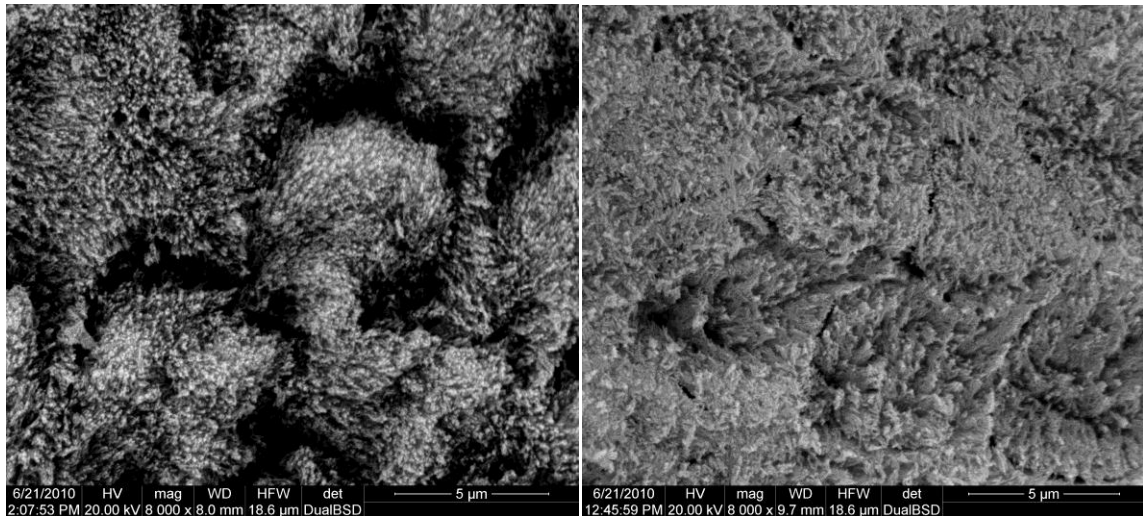


Figure 30: Etch surface morphology of tooth E at 8000 x (left is scrub and at right is passive application).

Scrub 20,000x

Passive 20,000x

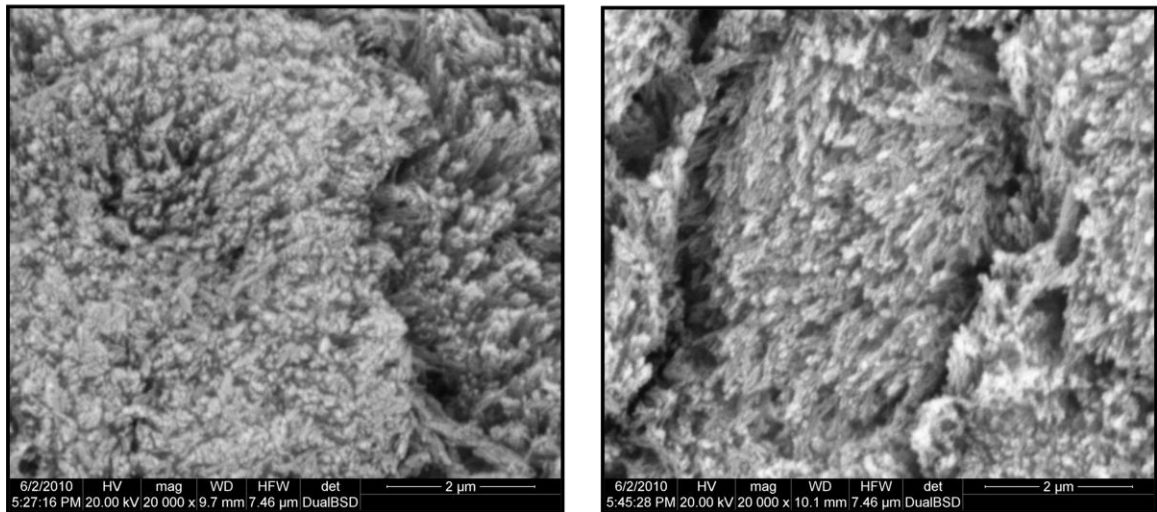


Figure 31: Etch surface morphology of tooth A at 20,000 x (left is scrub and at right is passive application).

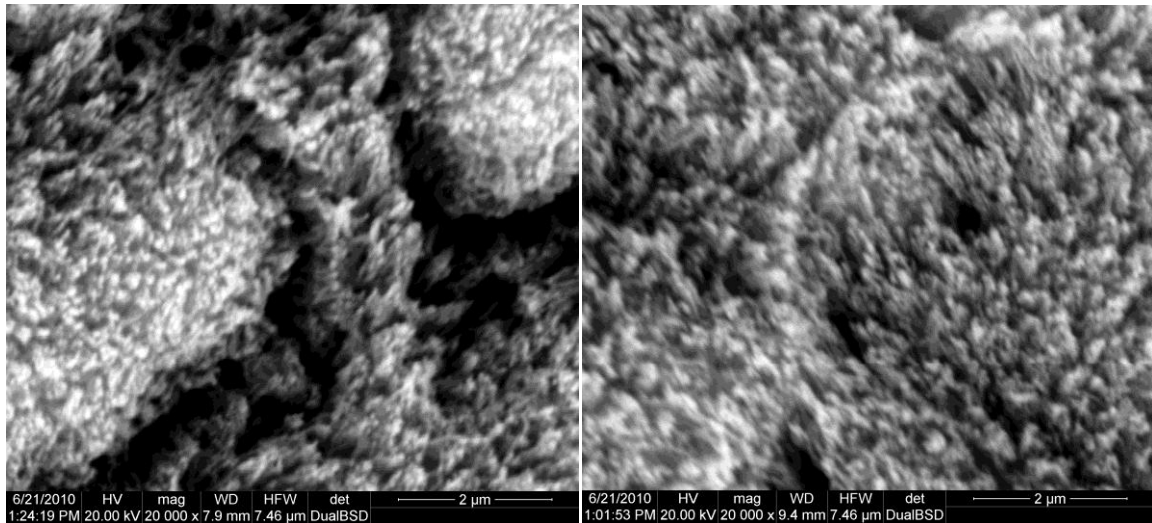


Figure 32: Etch surface morphology of tooth B at 20,000 x (left is scrub and at right is passive application).

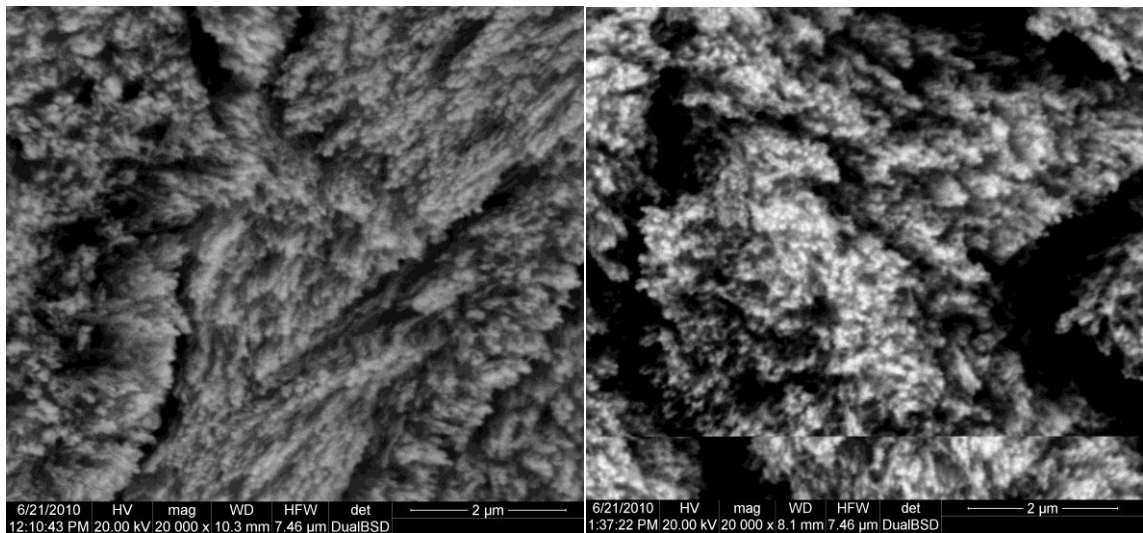


Figure 33: Etch surface morphology of tooth C at 20,000 x (left is scrub and at right is passive application).

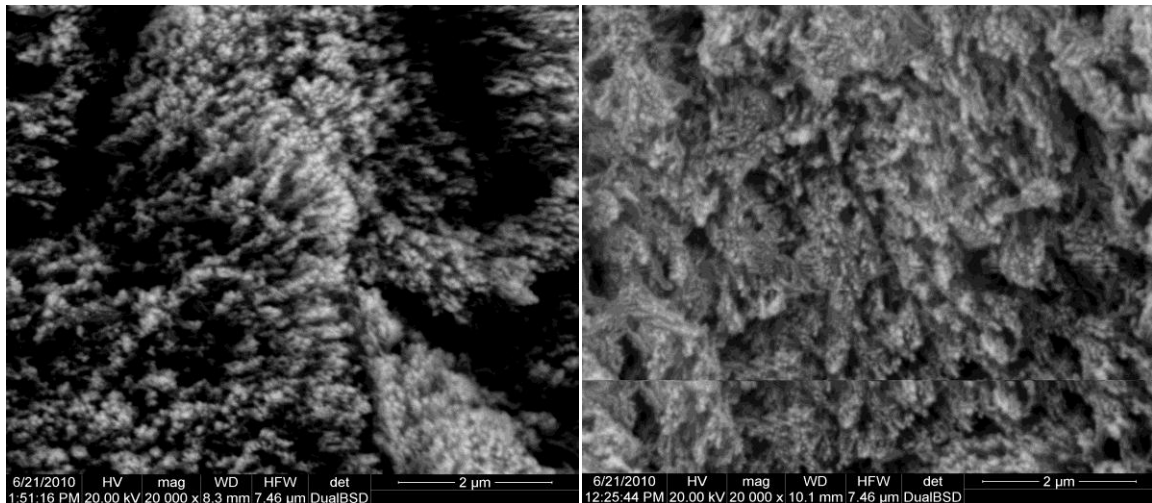


Figure 34: Etch surface morphology of tooth D at 20,000 x (left is scrub and at right is passive application).

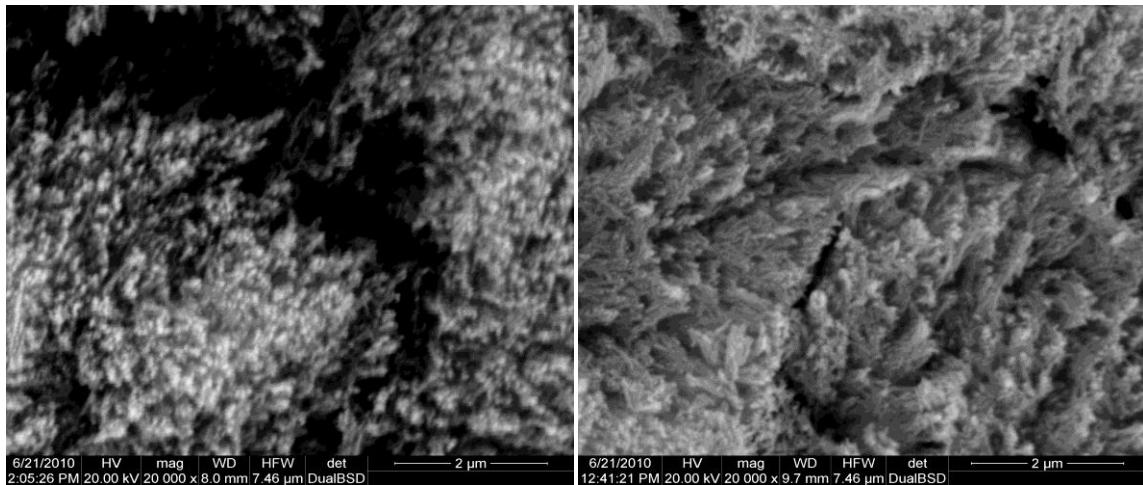


Figure 35: Etch surface morphology of tooth E at 20,000 x (left is scrub and at right is passive application).

APPENDIX C: IMAGES OF RESIN INFILTRATION

Resin infiltration images illustrate a dental restoration of composite resin to enamel surface where the cross-sectional view is shown.

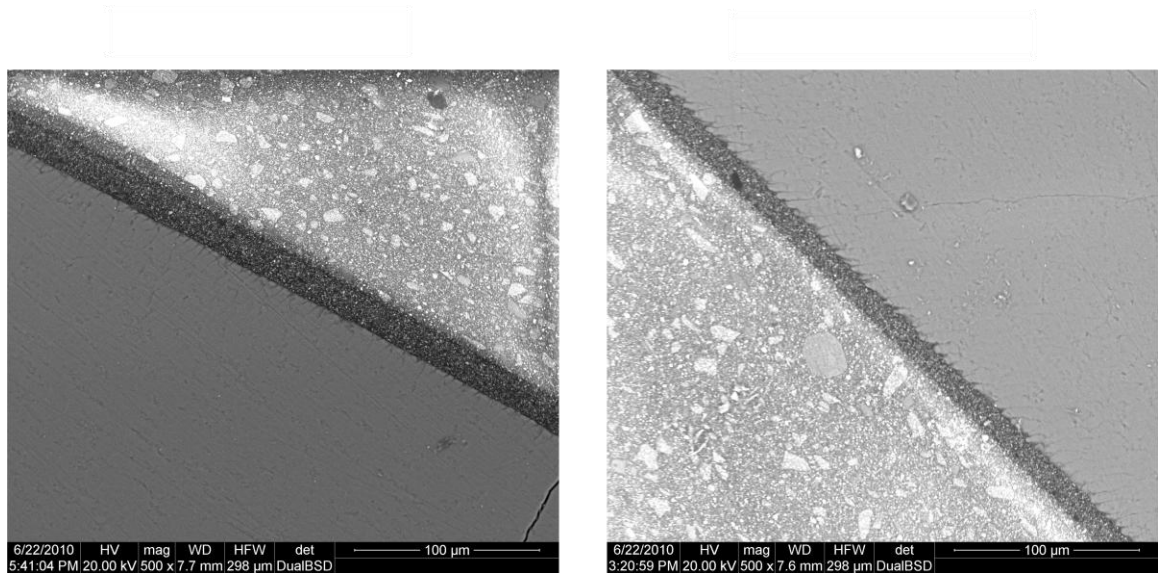


Figure 36: Resin infiltration of tooth A at 500x (at left is passive and at right is scrubbing application).

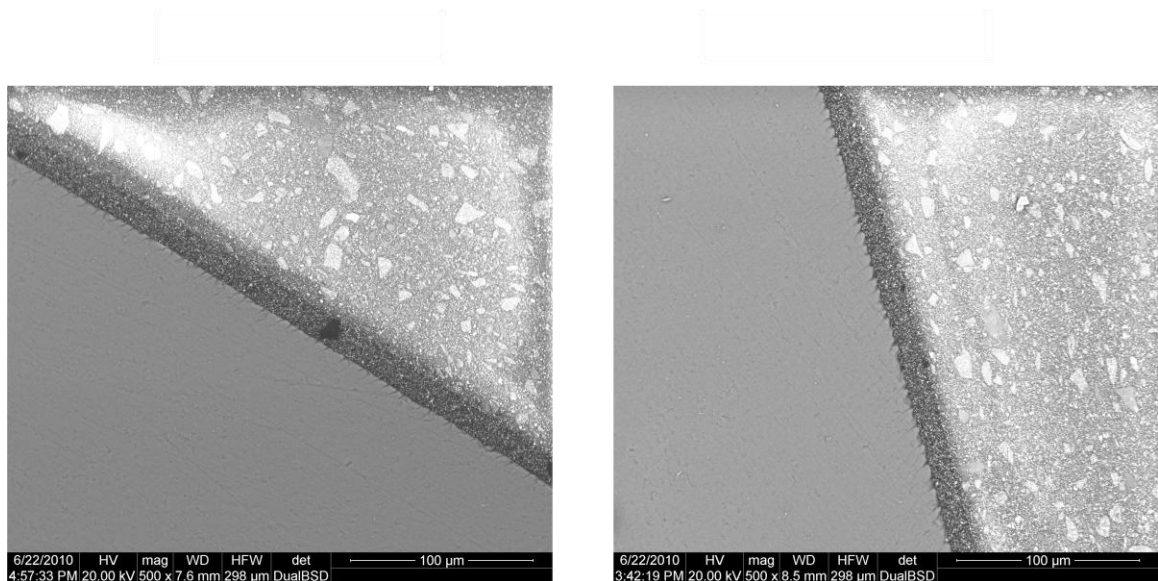


Figure 37: Resin infiltration of tooth B at 500x (at left is passive and at right is scrubbing application).

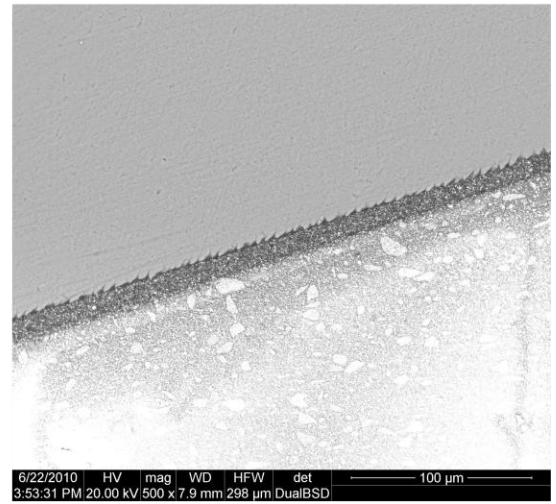
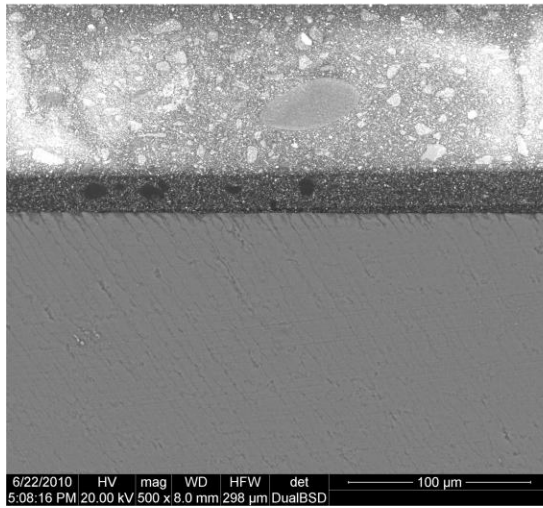


Figure 38: Resin infiltration of tooth C at 500x (at left is passive and at right is scrubbing application).

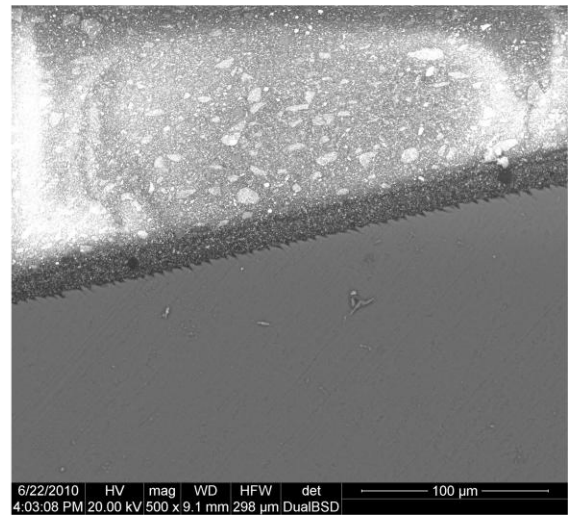
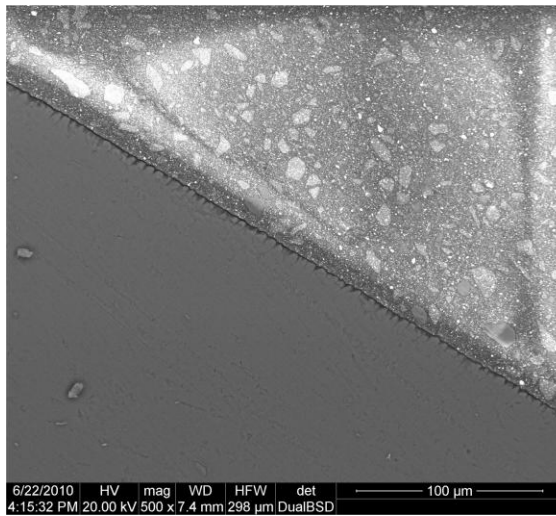


Figure 39: Resin infiltration of tooth D at 500x (at left is passive and at right is scrubbing application).

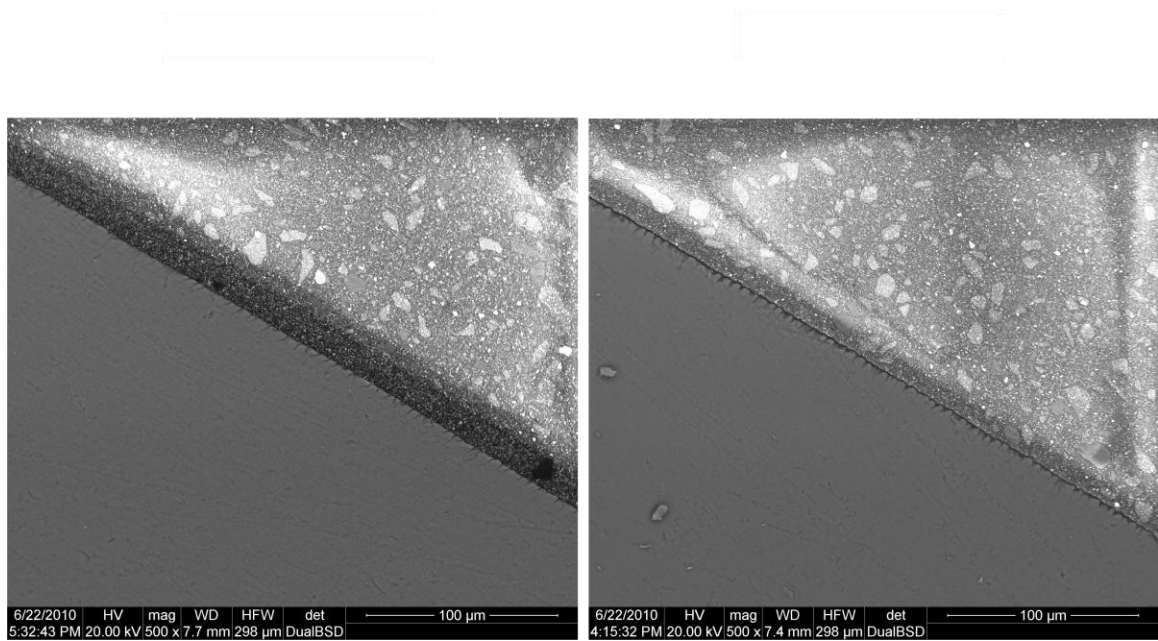


Figure 40: Resin infiltration of tooth E at 500x (at left is passive and at right is scrubbing application).

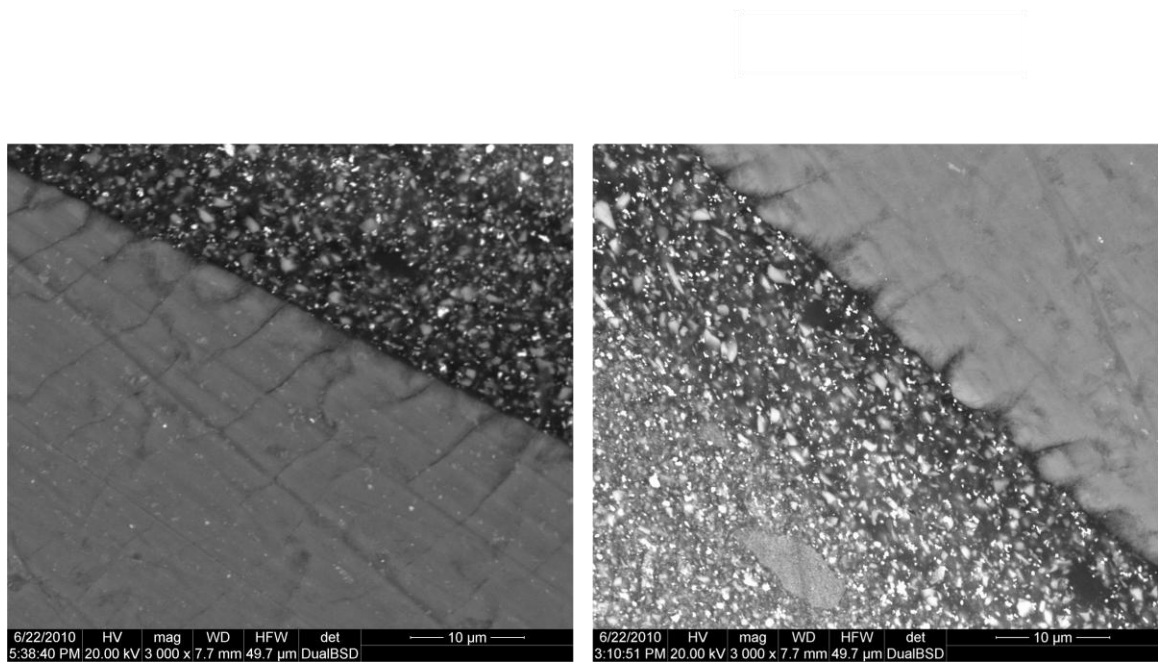


Figure 41: Resin infiltration of tooth A at 3000x (at left is passive and at right is scrubbing application).

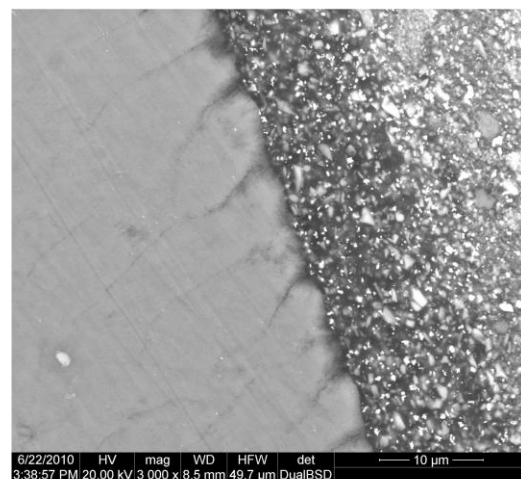
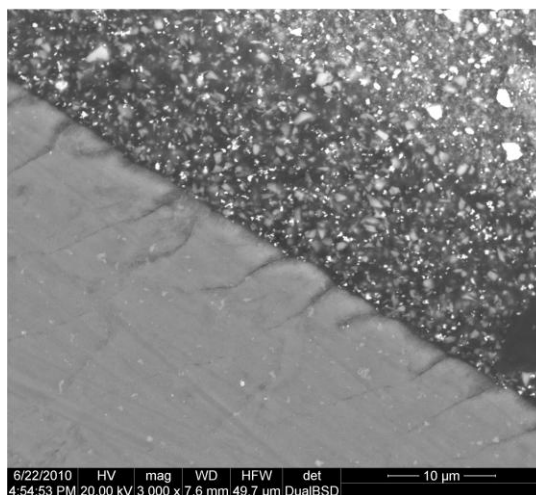


Figure 42: Resin infiltration of tooth B at 3000x (at left is passive and at right is scrubbing application).

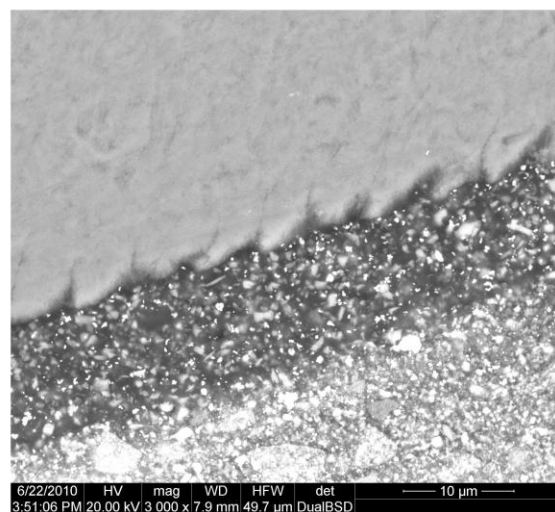
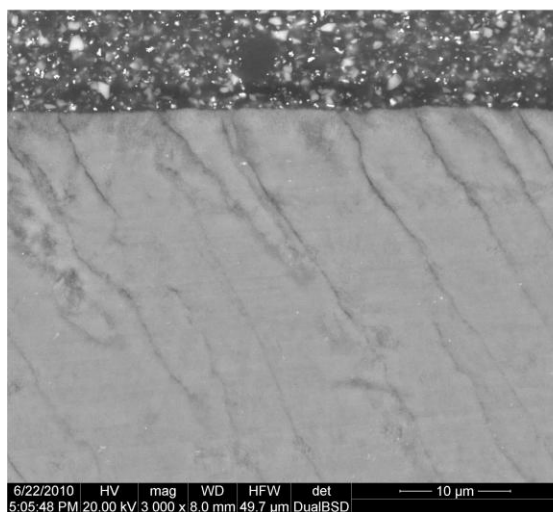


Figure 43: Resin infiltration of tooth C at 3000x (at left is passive and at right is scrubbing application).

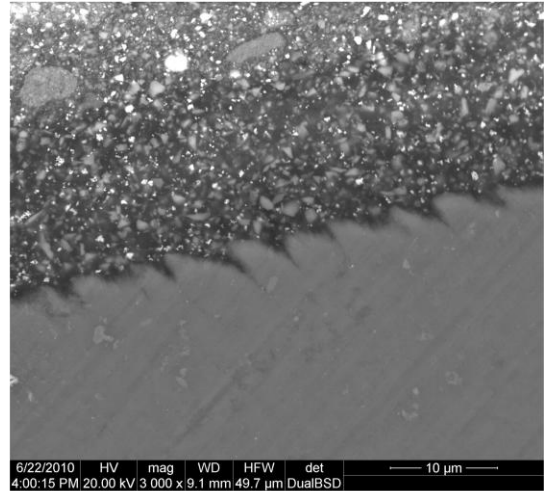
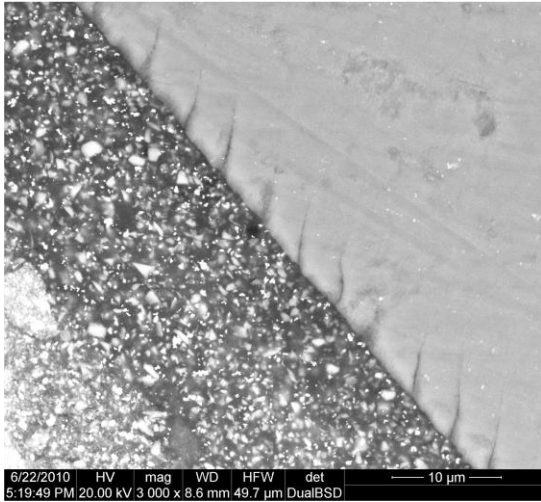
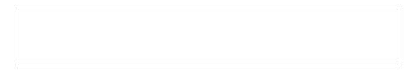


Figure 44: Resin infiltration of tooth D at 3000x (at left is passive and at right is scrubbing application).

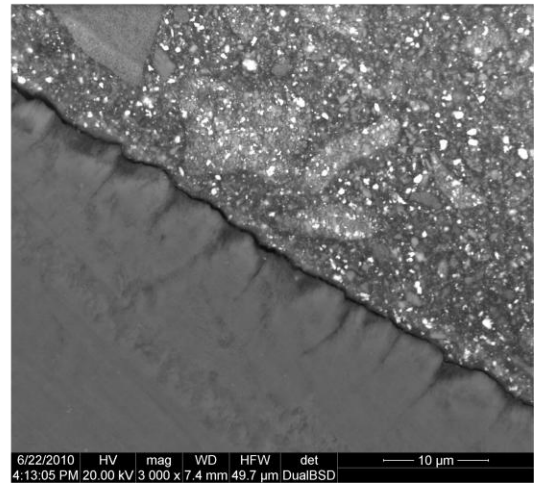
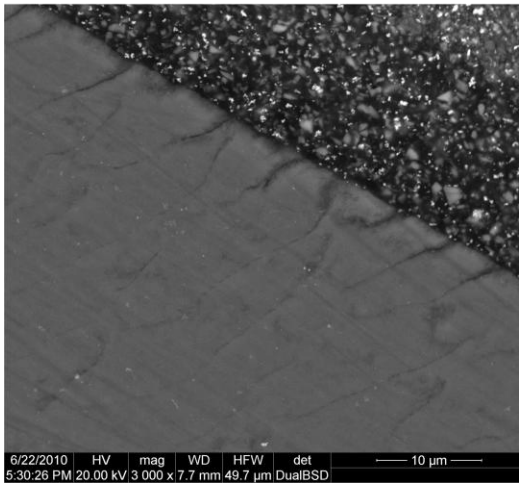
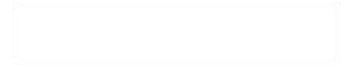


Figure 45: Resin infiltration of tooth E at 3000x (at left is passive and at right is scrubbing application).

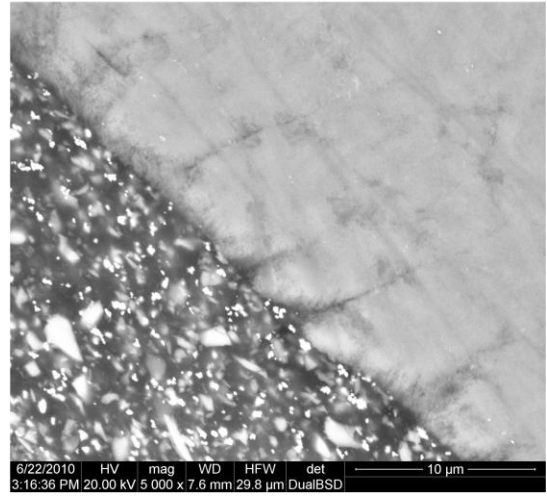
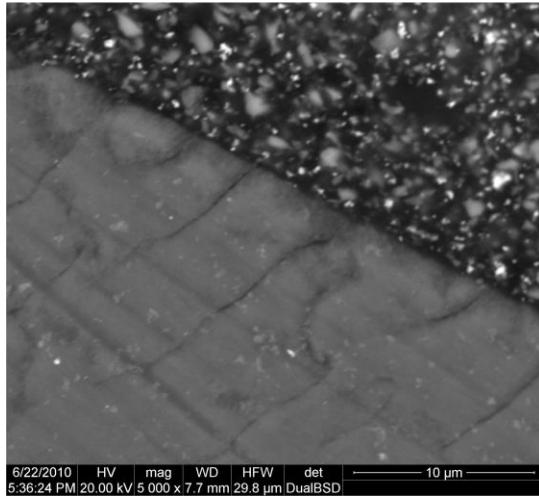


Figure 46: Resin infiltration of tooth A at 5000x (at left is passive and at right is scrubbing application).

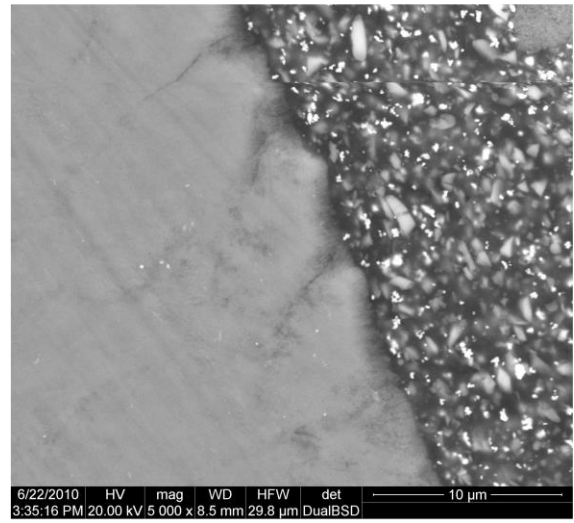
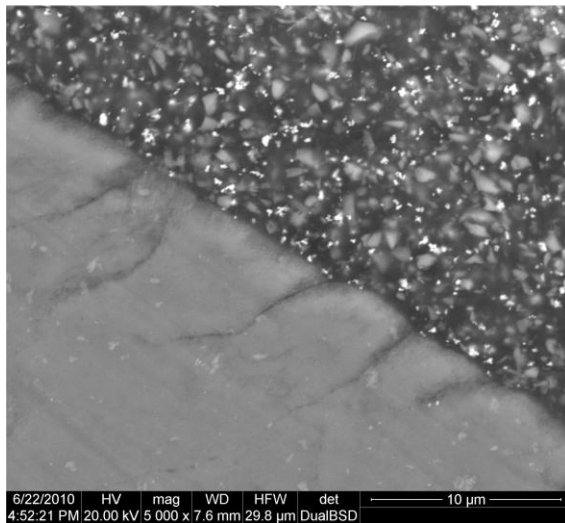


Figure 47: Resin infiltration of tooth B at 5000x (at left is passive and at right is scrubbing application).

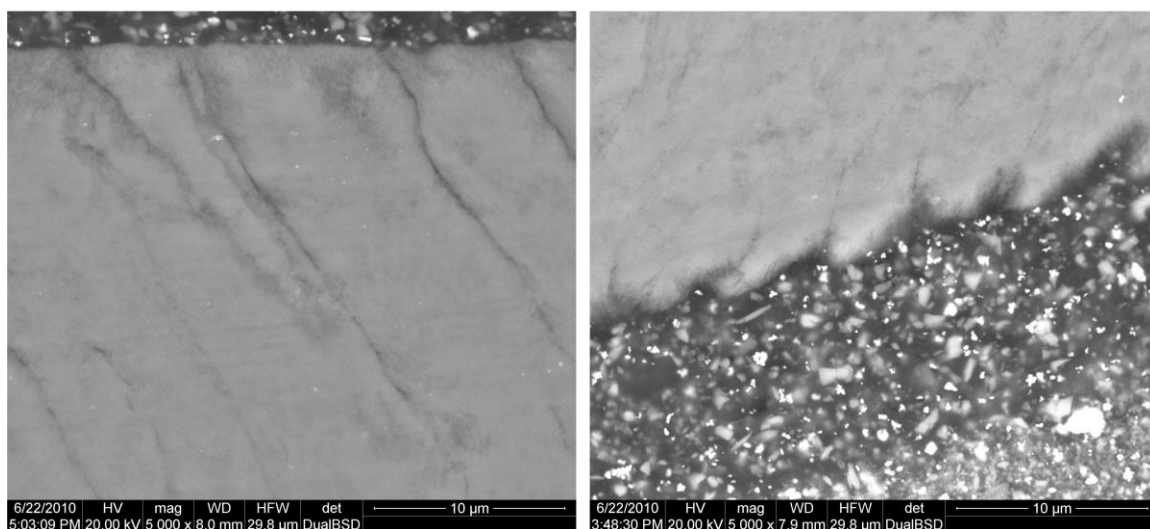


Figure 48: Resin infiltration of tooth C at 5000x (at left is passive and at right is scrubbing application).

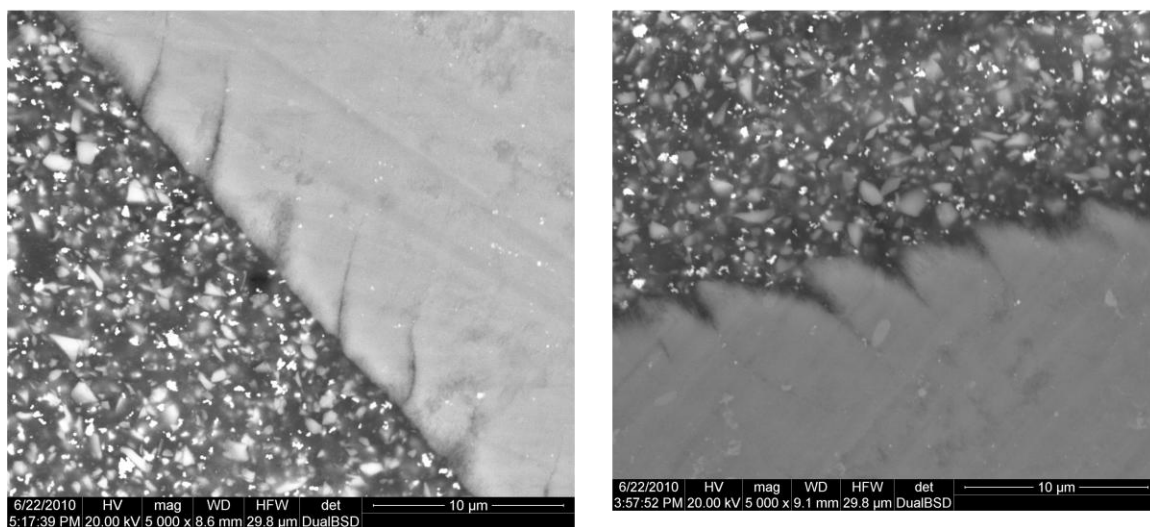


Figure 49: Resin infiltration of tooth D at 5000x (at left is passive and at right is scrubbing application).

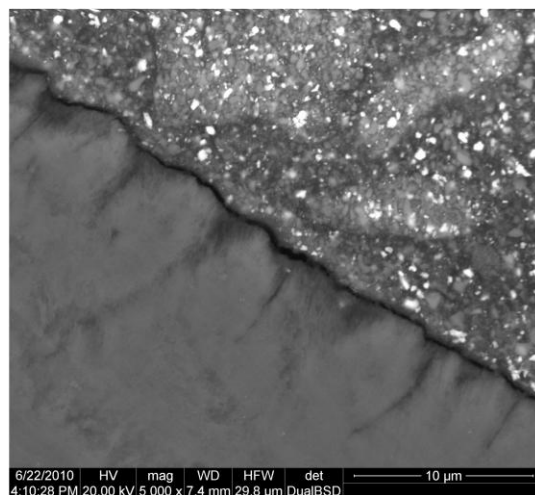
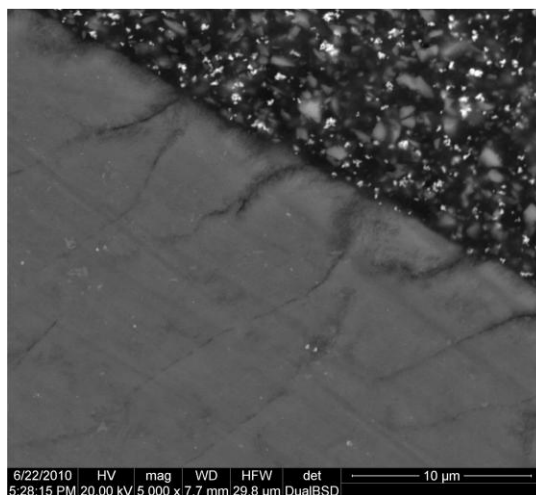


Figure 50: Resin infiltration of tooth E at 5000x (at left is passive and at right is scrubbing application).